(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 March 2001 (29.03.2001)

PCT

(10) International Publication Number
WO 01/20995 A1

(51) International Patent Classification[7]: A01N 57/00, C07D 305/00, 309/00, 311/00, 311/02, 311/04, 321/00

(21) International Application Number: PCT/US00/26177

(22) International Filing Date: 22 September 2000 (22.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/155,822 23 September 1999 (23.09.1999) US

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(81) **Designated States** *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** COMPOUNDS DIRECTED AGAINST PILUS BIOGENESIS AND ACTIVITY IN PATHOGENIC BACTERIA; METHODS AND COMPOSITIONS FOR SYNTHESIS THEREOF

(57) **Abstract:** Many Gram-negative pathogens assemble adhesive structures on their surfaces that allow them to colonize host tissues and cause disease. Novel compositions which inhibit or prevent the formation of a pilus chaperone-subunit complex are disclosed. Interfering with the function of the pili chaperone negatively affects the chaperone/usher pathway which is one molecular mechanism by which Gram-negative bacteria assemble adhesive pili structures and thus prevent or inhibit pilus assembly. Also provided are methods for the treatment or prevention of diseases caused by tissue-adhering pilus-forming bacteria by inhibiting the function of pilus chaperones. Also provided are pharmaceutical preparations capable of inhibiting or preventing the formation of a pilus chaperone-subunit complex. Also provided are methods of synthesizing the N-substituted amino acid compounds and compounds useful for the synthesis thereof. In particular, novel fluorinated linker compounds and methods of synthesis are provided. Methods for using the fluorinated linker compounds in methods of solid-phase synthesis of the N-substituted amino acid compounds are also disclosed.

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COMPOUNDS DIRECTED AGAINST PILUS BIOGENESIS AND ACTIVITY IN PATHOGENIC BACTERIA; METHODS AND COMPOSITIONS FOR SYNTHESIS THEREOF

This application claims priority to co-pending United States provisional patent application Ser. No. 60/155,822, filed September 23, 1999, incorporated herein by reference.

Field of Invention

The invention relates to novel compounds and compositions which inhibit or prevent bacterial growth and/or attachment by inhibiting or preventing pilus biogenesis. Further provided are fluorinated linker compositions bound to a solid support for solid phase synthesis of N-substituted amino acid compositions, as well as derivatives of carboxylic acids in general.

Background of the Invention

Many pathogenic Gram-negative bacteria such as *Escherichia coli, Haemophilus influenzae, Salmonella enteriditis, Salmonella typhimurium, Bordetella pertussis, Yersinia enterocolitica, Yersinia perstis, Helicobacter pylori and Klebsiella pneumoniae* assemble hair-like adhesive organelles called pili on their surfaces. Pili are thought to mediate microbial attachment, often the essential first step in the development of disease, by binding to receptors present in host tissues and may also participate in bacterial-bacterial interactions important in biofilm formation.

The prevention or inhibition of normal pilus assembly in Gram-negative bacterium impacts the pathogenicity of the bacterium by preventing the bacterium to infect host tissues. Uropathogenic strains of *E. coli* express type 1 and P pili that bind to receptors present in uroepithelial cells. Type 1 appear to be more common in *E. coli* causing cystitis whereas adhesive P pili are virulence determinants associated with pyelonephritic strains of *E. coli*.

Type 1 pili are adhesive fibers expressed in *E. coli* as well as in most of the Enterobacteriaceae family. They are composite structures in which a short tip fibrillar structure containing FimG and the FimH adhesin (and possibly the minor component FimF as well) is joined to a rod comprised predominantly of FimA subunits. The type 1 adhesin, FimH, binds D-mannose oligosaccharides present in glycolipids and glycoproteins. In uropathogenic *E. coli*, this binding event has been shown to play a critical role in bladder colonization and disease. Type 1 pilus biogenesis proceeds via a highly conserved chaperone/usher pathway that is involved in the assembly of over 25 adhesive organelles in Gram-negative bacteria.

P pili are adhesive organelles encoded by eleven genes in the *pap* (pilus associated with pyelonephritis) gene cluster found on the chromosome of uropathogenic strains of *E. coli*. Six genes encode structural pilus subunits, PapA, PapH, PapK, PapE, PapF and PapG. *See* S.J. Hultgren et al., Cell 73:887 (1993). The pilus is a heteropolymeric surface fiber with an adhesive tip and consists of two major sub-assemblies, the pilus rod and the tip fibrillum. The pilus rod is a thick rigid rod made up of repeating PapA subunits arranged in a right-handed helical cylinder whereas the tip fibrillum is a thin, flexible tip fiber extending from the distal end of the pilus rod and is composed primarily of repeating PapE subunits arranged in an open helical configuration. Two components of the tip fibrillum, PapK and PapF, act as adaptors. PapK is thought to link the pilus rod to the base of the tip fibrillum and regulates the length of the tip fibrillum: its incorporation terminates its growth and nucleates the formation of the pilus rod. PapF is thought to join the PapG adhesin to the distal end of the flexible tip fibrillum. The biogenesis of P pili occurs via the highly conserved chaperone/usher pathway.

Periplasmic chaperones are required for the assembly of these pili constructed from pilus subunits. In the absence of an interaction with the chaperone, pilus subunits aggregate and are proteolytically degraded. Two of the genes in the *pap* operon, *papD* and *papC* , encode the chaperone and usher, respectively. Chaperones such as PapD in *E. coli* are required to bind to pilus proteins imported into the periplasmic space, partition them into assembly component complexes and prevent non-productive aggregation of the subunits in the periplasm. *See* Kuehn M. J. et al., Proc. Natl. Acad. Sci. USA,88:10586 (1991). PapD is a periplasmic chaperone that mediates the assembly of P pili. Detailed structural analysis has revealed that the PapD chaperone is the prototype member of a conserved family of periplasmic chaperones in Gram-negative bacteria. Periplasmic chaperones, along with outer membrane ushers, constitute a molecular mechanism necessary for guiding biogenesis of adhesive organelles in Gram-negative bacteria. These chaperones function to cap and partition interactive subunits imported into the periplasmic space into assembly competent complexes, making non-productive interactions unfavorable.

PapD binds to each of the pilus subunit types as they emerge from the cytoplasmic membrane and escorts them in assembly-competent, native-like conformations from the cytoplasmic membrane to outer membrane assembly sites comprised of PapC. PapC has been termed a molecular usher since it receives chaperone-subunit complexes and incorporates, or ushers, the subunits from the chaperone complex into the growing pilus in a defined order.

The crystal structures of the PapD alone, or in complex with a peptide from the PapG adhesin, have been solved and refined to 2.5 and 3.0 Å, respectively. *See* Holmgren

and Branden, Nature, (1989) 342:248; Kuehn et al., Science, (1993) 268:1234. Recently, the structures of PapD-PapK and FimC-FimH complexes were solved by X-ray crystallography. *See* Sauer, et al. Science (1999) 285:1058; Choudhury et al., Science, (1999) 285:1062. The PapD molecule has two immunoglobulin-like domains oriented in an L shape to form a cleft at their interface. The chaperone cleft contains surface-exposed residues that are highly conserved. Each immunoglobulin-like domain has a β-barrel structure formed by two antiparallel β-pleated sheets with an overall topology similar to an immunoglobulin fold. All members of the periplasmic chaperone superfamily have a conserved hydrophobic core that maintains the overall features of the two domains. During pilus biogenesis, PapD binds to and caps interactive surfaces on pilus subunits and prevents their premature aggregation in the periplasm. The chaperone-subunit complexes are targeted to the outer membrane usher where subunits assemble in a specific order to form a pilus. Kuehn et al. have shown that the G_1 β-strand of PapD forms a β-zipper interaction with the highly conserved COOH-terminal motif of pilus subunits. *See* Kuehn et al., Science, (1993) 268:1234. This COOH-terminal motif also comprises at least part of a primary surface for subunit-subunit assembly interactions, indicating that the direct capping of a primary assembly surface is part of the molecular basis by which periplasmic chaperones prevent the premature oligomerization of pilus subunits. In addition, the β-zipper interaction has been proposed to facilitate the folding of the subunit into a native-like conformation via a template-mediated mechanism.

In the absence of an interaction with the chaperone, pilus subunits aggregate and are proteolytically degraded. Kolmer et al. and Jones et al. have shown that the DegP protease is greatly responsible for the degradation of pilin subunits in the absence of the chaperone (*J. Bacteriol.* 1996, 178:5925; *BIBO* 1997, 16:6394). This discovery led to the elucidation of the fate of pilus subunits expressed in the presence or absence of the chaperone using monospecific antisera in Western blots of cytosolic membrane, outer membrane and perplasmic proteins prepared according to methods known in the art.

Thus, prevention or inhibition of normal pilus assembly in Gram-negative bacterium impacts the pathogenicity of the bacterium by preventing the bacterium to infect host tissues. Moreover, changes in the binding between pilus subunits and chaperones can have a dramatic impact on the efficiency of pilus assembly, and thus on the ability of Gram-negative bacterium to adhere to and consequentially, infect host tissues. Drugs that interfere with the assembly of pili should effectively disable pathogens responsible for a wide variety of gram-negative infections, such as those responsible for bladder, kidney and middle ear infections as well as food poisoning, gastric ulcers, diarrhea, meningitis, and other illnesses. Drugs that interfere with the assembly of pili are known collectively as pilicides.

Accordingly, a need exists, in general, for compounds and compositions which prevent or inhibit the interaction between pilus subunits and a chaperone as well as methods for utilizing such compounds and compositions for the treatment or prevention of bacterial infections or bacterial colonization.

Combinatorial chemistry has become a powerful tool for drug discovery in the pharmaceutical and biotechnology industries. Generally, combinatorial chemistry is defined as the repetitive and systematic covalent attachment of different structural moieties to one another to produce a mixture of numerous distinct molecular entities or target molecules (*i.e.*, combinatorial libraries); desired target molecules include peptides, oligonucleotides, and small organic molecules. Combinatorial chemistry is frequently utilized to generate a group of structurally related analogs which can then be evaluated to establish structure-activity relationships (SAR) and to optimize biological potency. *See, e.g.*, M. A. Gallop et al., *J. Med. Chem.*, 37:1233-1248 (1994).

Solid-phase synthesis was developed into a fast and reliable technique for synthesis of peptides almost 40 years ago. During the last ten years interest in solid-phase organic synthesis has increased substantially due to the emergence of combinatorial and parallel synthesis strategies that are now being widely applied in pharmaceutical research. This has brought about a need for adoption of synthetic organic methodology developed in solution so as to become compatible with various solid-supports.

NMR spectroscopy is a well established technique in solution-phase organic chemistry and appears to be the analytical tool of choice also for solid-phase organic synthesis. However, conventional ^{1}H and ^{13}C NMR spectra of substances attached to a solid support usually suffer from drawbacks such as inhomogeneous line broadening, prolonged spectral acquisition and interference of signals from the solid support. To circumvent these problems, several techniques for structural elucidation have been developed including magic angle spinning, use of selectively ^{13}C enriched building blocks, presaturation of support signals, and combinations of these techniques. However, high costs and/or requirements for specialized instrumentation are drawbacks associated with these methods.

An additional method used to address the problems with interference of signals from the solid support is to substitute ^{1}H or ^{13}C for another nucleus, such as ^{19}F, which is not part of commonly used solid-supports. ^{19}F NMR spectroscopy is almost as sensitive as ^{1}H NMR spectroscopy since the natural abundance of ^{19}F is 100%. Another advantage of ^{19}F is that the large polarizability of the fluorine electron cloud makes it sensitive to remote changes in electron density, thereby spreading ^{19}F resonances over a large range of chemical shifts. These features render fluorine well-suited as a sensor for monitoring solid-phase chemical conversions using gel-phase ^{19}F NMR spectroscopy. The choice of the

linker is an important aspect in all solid-phase synthesis and it would be an advantage if the linker can serve analytical purposes. Accordingly, fluorine-derivatized linkers or solid supports should be useful for quantitative monitoring of solid-phase organic synthesis.

Accordingly, compositions and methods of synthesis are needed for synthesizing pilicidal compositions. Specifically, compositions and methods are needed which allow for the monitoring of reactions performed on solid-phase as well as determination of the structures of the resulting products while still linked to the solid support.

Summary of the Invention

Accordingly, among the objects of the invention, therefore, may be noted:

(i) The provision of antibacterial compositions capable of inhibiting or preventing pilus assembly in a Gram-negative bacterium and libraries of such antibacterial compositions.

(ii) The provision of processes for synthesizing such antibacterial compounds in solution and on solid-phase.

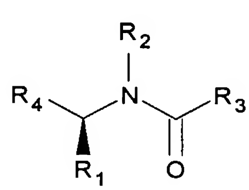
(iii) The provision of methods of using such antibacterial compositions for treating Gram-negative infections; methods of preventing or inhibiting the attachment of Gram-negative organisms to host tissues; methods of preventing or inhibiting biofilm formation; and methods of preventing or inhibiting bacterial colonization by a Gram-negative organism.

(iv) The provision of linker compounds which may be used in the synthesis of the antibacterial compounds and method of synthesizing the linker compounds.

(v) The provision of methods of monitoring the synthesis of the antibacterial compounds and libraries of such antibacterial compounds on solid phase using the linker compounds.

(vi) The provision of complexes of the antibacterial compounds complexed to the linker compounds which are affixed to a solid support.

Briefly, therefore, the present invention is directed to a compound having the formula:

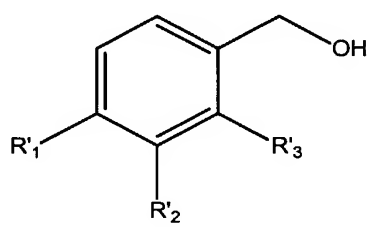


wherein each of R_1, R_2 and R_3 is independently a substituted or unsubstituted alkyl (C_{1-10}), substituted or unsubstituted acyl (C_{2-15}), substituted or unsubstituted aryl (C_{6-14}), substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl (C_{7-15}),

substituted or unsubstituted heteroarylalkyl or substituted or unsubstituted heterocycloalkyl;R_4 is a carboxy ($-CO_2H$), carboxamide ($-CONH_2$), aldehyde (-CHO), boronate ($-B(OH)_2$), phosphonate ($PO(OH)_2$) or ketone (-COR) wherein R is a halogenated or unsubstituted alkyl (C_{1-3}); and the salts, esters and amines thereof.

Another object of the invention is to provide antibacterial compounds and pharmaceutical compositions containing such antibacterial compounds which have broad specificity for a diverse group of Gram-negative bacteria. A further object of this invention is to provide compounds and methods for preventing and inhibiting biofilm formation which comprise administering an effective amount of such compounds to an environment or surface containing Gram-negative bacteria. Additionally, among other objects of the invention is to provide methods for utilizing such compounds, such as methods of treating or preventing Gram-negative infections which comprise providing to a subject an effective amount of the above compositions.

Another aspect of the present invention is to provide fluorinated linkers for use in the synthesis of the N-substituted amino acid derivatives on solid phase. Thus, the present invention is also directed to linker compounds having the formula:



wherein R'_1 is $-CO_2H$, $-(CH_2)_nCO_2H$ or $-O(CH_2)_nCO_2H$ wherein n is between 1 and 10, preferably n is between 1 and 5, and more preferably, n is 1 or 2; and R'_2 and R'_3 is independently fluorine or hydrogen provided that when either R'_2 or R'_3 is fluorine, the other is hydrogen. Preferably, R'_2 of the linker compound is hydrogen and R'_3 of the compound is fluorine.

A related object of the present invention is to provide processes of synthesis of the above linker compounds. These processes include the steps of hydrolyzing one of the ester moieties of dimethyl-2-fluoroterephtalate, reducing the remaining ester and separating the two regioisomers. Alternatively, another process of synthesis includes (a) dealkylating a 2-fluoro-4-propoxybenzoic acid; (b) reducing the carboxylic acid of the product of step (a) thereby producing a hydroxymethylphenol compound; (c) alkylating the phenolic hydroxyl group of the hydroxymethylphenol compound; and (d) hydrolyzing the product of step (c) under basic conditions.

It is another object of the invention is to provide processes for the synthesis of the antibacterial compounds in solution and on solid phase. The process of synthesis of such compounds includes the steps of condensing a compound 6 of Reaction Scheme 1 with a

salicyaldehyde selected from the group consisting of the reagent compounds listed in Table A herein. Preferably, the antibacterial compounds are synthesized on solid phase using the linker compounds of the invention. Such solid phase synthesis of the compounds include the steps of affixing a linker compound to a solid support to give a benzylic alcohol; subjecting the benzylic alcohol to acylation with bromoacetic acid; subjecting the bromoacetate to a nucleophilic substitution with an amine; acylating with ethyl malonyl chloride thereby forming a N-alkyl-N-(malonamic acid ethyl ester)-glycine derivative; condensing the N-alkyl-N-(malonamic acid ethyl ester)-glycine derivative with a salicyaldehyde; and cleaving the compound from the linker compound under acidic or basic conditions.

Another process of solid phase synthesis of the antibacterial compounds provided by the present invention includes the steps of (a) affixing a linker compound onto a solid support to give a benzylic alcohol; (b) coupling a Fmoc-protected amino acid to the benzylic alcohol thereby producing an amino acid functionalized resin; (c) removing the α-amino group of the product of step (b); (c) alkylating the α-amino group of the product of step (c) by reductive alkyation; (d) removing excess aldehyde from the amino acid functionalized resin; (e) acylating with an acid chloride thereby producing a N-(alkylated)-N-(acylated)-amino acid derivative; and (f) cleaving the compound from the linker under acidic or basic conditions.

A further object of the present invention is to provide an improved method of synthesizing a combinatorial library wherein the improvement comprises affixing a linker compound of the present invention onto a solid support. This library of compounds synthesized using this improved synthesis can be used to create a library of compounds which can be screened for antibacterial activity.

Yet another object of the present invention is to provide methods of monitoring solid-phase synthesis of the antibacterial compounds. The process includes the steps of affixing a linker compound onto a solid support, utilizing a means for measuring a signal originating from the linker compound and utilizing the signal as an internal reference thereby enabling the monitoring of reactions of said solid-phase synthesis of compounds. Preferably, the signal originating from the linker compounds will be a ^{19}F resonance and is measured using ^{19}F NMR spectroscopy.

Another related object of the present invention is to provide complexes of the antibacterial compositions complexed to the fluorine linker compounds which are affixed to a solid support.

Other objects and features will be in part apparent and in part pointed out hereinafter.

Description of the Figures

Figure 1 represents ^{19}F NMR spectra showing (a) resin-bound linker 2, (b) that ~ 90% conversion of 2 into 3 was obtained by acylation using 3 eq. of bromoacetic acid, (c) complete transformation of 2 into 3 was obtained after repeating the acylation with 1.5 eq. of bromoacetic acid, and (d) 8 after Knoevenagel condensation of 6 with salicylaldehyde and piperidine, indicating that ~20% of the product was cleaved form the resin during the condensation.

Figure 2 represents ^{19}F NMR spectra of resin-bond products obtained after reductive alkylation of 40 using the reaction conditions given in entries 1-3, Table D. The ^{19}F resonance at d -115 ppm originates from the linker and shows that coupling of Fmoc-Phe-OH to 2 was quantitative. This resonance was used as internal standard. Integration of the ^{19}F resonance originating from the *p*-fluorobenzyl residue (d -116 ppm) showed that: (a) the reaction conditions in entry 1 resulted in ~64% alkylation of 40, (b) the conditions in entry 2 led to ~55% alkylation, and (c) those in entry 3 gave ~78% alkylation of 40.

Figure 3 is a gel-phase ^{19}F NMR spectroscopy of resin-bound products obtained after reductive alkylation of 40, as described in entries 4 - 6, Table D, and subsequent acylation with 4-fluoronaphthoyl chloride. The line-broadening in the spectra is due to rotamers about the amide bond in 32a. Integration over the ^{19}F resonances revealed that: (a) use of 1.5 eq. *p*-F-benzaldehyde (entry 4) resulted in ~8% dialkylation, (b) increasing the excess of *p*-F-benzaldehyde to 3 eq. (entry 5) gave slightly increased formation of dialkylated 42a (~10%), whereas (c) removing excess aldehyde prior to addition of $NaBH_3CN$ (entry 6) proved to be the most efficient method, which reduced the formation of 42a to <2% and increased the overall yield of 43b to ~92%.

Figure 4 is a gel-phase ^{19}F NMR spectra of 43b obtained after reductive alkylation of 40 with 4,4,4-trifluorobutyraldehyde under the following conditions: (a) 4,4,4-trifluorobutyraldehyde (3 eq.) with direct addition of $NaBH_3CN$ gave 43b in 48%, whereas (b) removal of excess aldehyde prior to the addition of $NaBH_3CN$ increased the yield of 43b to 66%. The resins were acylated with 4-fluoronaphthoyl chloride after the reductive alkylation.

Abbreviations and Definitions

The terms and abbreviations have the indicated meaning as used herein.

The term “alkyl" is intended to include linear, branched, or cyclic hydrocarbon structures and combinations thereof. "Lower alkyl" means alkyl groups of from 1 to 8 carbon atoms. Examples of lower alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, s-and t-butyl, pentyl, hexyl, octyl, cyclopropylenthyl, bornyl and the like.

The "alkenyl" includes C_2-C_8 unsaturated hydrocarbons of a linear, branched, or cyclic (C_5-C_6) configuration and combinations thereof. Examples of alkenyl groups

include vinyl, allyl, isopropenyl, pentenyl, hexenyl, c-hexenyl, 1-propenyl, 2-butenyl, 2-methyl-2-butenyl and the like.

The term "alkynyl" includes C_2-C_8 hydrocarbons of a linear or branched configuration and combinations thereof containing at least one carbon-carbon triple bond. Examples of alkynyl groups include ethyne, propyne, butyne, pentyne, 3-methyl-1-butyne, 3,3-dimethyl-1-butyne and the like.

The term "alkoxy" refers to groups of from 1 to 8 carbon atoms of a straight, branched, cyclic configuration and combinations thereof. Examples include methoxy, ethoxy, propoxy, isopropoxy, cyclopropyloxy, cyclohexyloxy and the like.

The term "acyl" refers to the group --C(O)-Z', where Z is a lower alkyl. As used herein, "lower alkyl" means alkyl groups of from 1 to 8 carbon atoms. Examples of lower alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, s-and t-butyl, pentyl, hexyl, octyl, cyclopropylenthyl, bornyl and the like.

The term "acylamino" refers to acylamino groups of from 1 to 8 carbon atoms of a straight, branched or cyclic configuration and combinations thereof. Examples include acetylamino, butylamino, cyclohexylamino and the like.

The term "aryl" and "heteroaryl" mean a five or six-membered aromatic or heteroaromatic ring containing zero to three heteroatoms selected form O, N, and S; a bicyclic nine or ten-membered aromatic or heteroaromatic ring system containing zero to three heteroatoms selected from O, N, and S; or a tricyclic thirteen or fourteen-membered aromatic or heteroaromatic ring system containing zero to three heteroatoms selected from O, N, and S.

The term "arylalkyl" means an alkyl residue attached to an aryl ring. Examples include, e.g., benzyl, phenethyl and the like.

The term "carbonyl" refers to the group -CO. Examples of organic carbonyl compounds are ketones, aldehydes, carboxylic acids and the like.

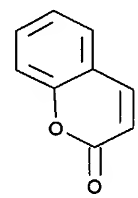
The term "heteroarylalkyl" means an alkyl residue attached to a heteroaryl ring. Examples include, pyridinylmethyl, pyrimidinylethyl and the like.

The term "heterocycloalkyl" means a cycloalkyl where one to two of the methylene (CH_2) groups is replaced by a heteroatom such as O, NR' (wherein R' is H or alkyl), S or the like; with the proviso that when two heteroatoms are present, they must be separated by at least two carbon atoms. Examples of heterocycloalkyls include tetrahydropyranyl, piperidynl, dioxanyl and the like.

The term "hydrocarbyl" is meant a monovalent substituent containing only carbon and hydrogen which may be straight or branched chain, saturated or unsaturated, aromatic or nonaromatic or both and can be cyclic or noncyclic. An example of a hydrocarbyl alcohol of 1-10C could include cyclopentyl ethyl alcohol, 2-pentyl alcohol, 3-butynyl alcohol, 2,4-dimethyl hexyl alcohol, benzyl alcohol and the like.

The term "carboxyalkyl" means --C(O)R", wherein R" is alkyl.

As used herein, "coumarin" shall refer to the following structure:



The term "substituted" alkyl, alkenyl, alkynyl, cycloalkyl, or heterocycloalkyl means alkyl, alkenyl, alkynyl, cycloalkyl or heterocycloalkyl wherein up to three H atoms on each C atom therein are replaced with halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy, carboxamido, cyano, carbonyl, NO_2, NR' R" (wherein each R' and R" is H, alkyl or arylalkyl), alkylthio, alkylthiol, sulfoxide, sulfone, acylamino, amidino, phenyl, benzyl, heteroaryl, phenoxy, benzyloxy, heteroaryloxy, and substituted phenyl, benzyl, heteroaryl, phenoxy, benzyloxy or heteroaryloxy.

As used herein "natural number" means a positive number including zero.

In general the abbreviations used herein for designating the amino acids are based on recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature. *See* Biochemistry, 11, 1732 (1972). For instance Ala, Leu and Gly represent the amino acid residue of L-alanine, L-leucine and glycine, respectively. The term "residue" means a radical derived from the corresponding L-amino acid by eliminating the hydroxy portion of the carboxy group and a hydrogen of the α-amino group. The term "amino acid side chain" is that part of an amino acid exclusive of the $--CH(NH_2)COOH$ portion, as defined by K. D. Kopple, "Peptides and Amino Acids", W. A. Benjamin Inc., New York and Amsterdam, 1966, pgs 2 and 33. Examples of amino acid side chains are $-CH_2-CH(CH_3)_2$ (the side chain of leucine), -H (side chain of glycine), $-CH_3$ (the side chain of alanine), $-CH_2CONH_2$ (the side chain of asparagine), $-CH_2SH$ (the side chain of cysteine), 2-(3-indolyl)-ethyl (the side chain of tryptophan), $-CH_2CH_2SCH_3$ (the side chain of methionine), $-CH_2OH$ (the side chain of serine), 4-hydroxybenzyl (the side chain of tyrosine), $-CH(CH_3)_2$ (the side chain of valine), benzyl (the side chain of phenylalanine), $-CH(CH_3)CH_2CH_3$ (the side chain of isoleucine), $-CH(OH)CH_3$ (the side chain of threonine) and the like. Note, therefore, that the term "amino acid side chain" includes hydrogen.

The amino acids and amino acid residues are of the L or D configuration. It will be noted that the structures of the compounds of this invention include asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry are included within the scope of this invention. Such isomers are obtained in substantially

pure form by classical separation techniques and by sterically controlled synthesis and have arbitrarily been named as isomers L or D, respectively.

The terms "hydrophobic amino acid" and "hydrophobic amino acid residue" as used interchangeably herein means the common amino acids and amino acid residues having hydrophobic aromatic or aliphatic side chains which include tyrosine, tryptophan, phenylalanine, histidine, valine, cysteine, leucine, isoleucine, alanine, glycine, and methionine.

The terms "polar amino acid" and "polar amino acid residue" as used interchangeably herein means common amino acids and amino acid residues having polar side chains which include serine, threonine, glutamine and asparagine.

The terms "charged amino acid" and "charged amino acid residue" as used interchangeably herein means the common amino acids and amino acid residues having charged side chains which include lysine, arginine, aspartic acid and glutamic acid.

As utilized herein, the term "pilus" or "pili" relates to fibrillar heteropolymeric structures embedded in the cell envelope of many tissue-adhering pathogenic bacteria, notably pathogenic gram negative bacteria. In the present specification, the terms pilus and pili will be used interchangeably. A pilus is composed of a number of "pilus subunits" which constitute distinct functional parts of the intact pilus.

The term "chaperone" relates to a molecule in living cells which bind to pili subunits during the assembly of pili structures. Many molecular chaperones are involved in the process of pilicide biogenesis. Specialized molecular chaperones are "periplasmic chaperones" which are bacterial molecular chaperones exerting their main actions in the "periplasmic space." The periplasmic space constitutes the space in between the inner and outer bacterial cell membrane. Periplasmic chaperones are involved in the process of correct assembly of intact pili structures. When used herein, the use of the term "chaperone" designates a molecular, periplasmic chaperone unless otherwise indicated.

The phrase "preventing or inhibiting binding between pilus subunits and a periplasmic chaperone" indicates that the normal interaction between a chaperone and its natural ligand, *i.e.*, the pilus subunit, is being affected either by being inhibited, expressed in another manner, or reduced to such an extent that the binding of the pilus subunit to the chaperone is measurably lower than is the case when the chaperone is interacting with the pilus subunit at conditions which are substantially identical (with regard to pH, concentration of ions, and other molecules) to the native conditions in the periplasmic space. Measurement of the degree of binding can be determined *in vitro* by methods known to the person skilled in the art (microcalorimetry, radioimmunoassays, enzyme based immunoassays, surface-plasmon resonance, etc.).

The term "linker" refers to any molecule containing a chain of atoms, *e.g.*, carbon, nitrogen, oxygen, etc., that serves to link the molecules to be synthesized on the support

with the support. The linker is usually attached to the support via a covalent bond, before synthesis on the support starts, and provides one or more sites for attachment of precursors of the molecules to be synthesized on the support. Various linkers can be used to attach the precursors of molecules to be synthesized to the solid phase support.

The term "solid support" refers broadly to supports used in the solid phase synthesis of, for example, peptides, nucleic acids, oligonucleotides, and small organic molecules. Solid supports include, but are not limited to, polymer resins (e.g., polyethylene glycol and polystyrene), gels (e.g., polyethylene glycol gels), polyacrylamide/polyethylene glycol copolymer resins, controlled pore glass supports (e.g., the CPG supports commercially available from Millipore), and silica beads and wafers.

The term “antibodies” also includes any immunologically reactive fragment of the immunoglobulins such as Fab, Fab’ and F(ab’)2 fragments as well as modified immunoreactive forms such as Fv regions, which are produced by manipulation of the relevant genes.

The term “treatment” includes both prophylaxis and therapy. Thus, in treating a subject, the compounds of the invention may be administered to a subject already harboring a bacterial infection or in order to prevent such infection from occurring.

The phrase “an effective amount” means an amount of the substance in question which will in a majority of subjects have either the effect that the disease caused by the pathogenic bacteria is cured or, if the substance has been given prophylactically, the effect that the disease is prevented from manifesting itself. The term “an effective amount” also implies that the substance is given in an amount which only causes mild or no adverse effects in the subject to whom it has been administered, or that the adverse effects may be tolerated from a medical and pharmaceutical point of view in the light of the severity of the disease for which the substance has been given.

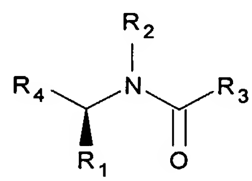
The phrase “subject in need thereof” means in the present context a subject, which can be any animal, including a human being, who is infected with, or is likely to be infected with, tissue-adhering pilus-forming bacteria which are believed to be pathogenic.

Detailed Description

The present invention is directed to a novel class of compounds which are effective in preventing or inhibiting pilus biogenesis and activity. The compounds of the invention may be effective in treating, preventing and inhibiting bacterial infections caused by Gram-negative organisms. Further, the present invention is directed to methods of utilizing such pilicidal compounds and to processes and compositions useful for the synthesis of such pilicidal compounds. The compounds of this invention exert their effects by interfering with the function of pilus chaperones to form pili from pilus subunits in the periplasm of the bacterium. Specifically, the compounds of the present invention

inhibit or prevent the formation of the complex between PapD and PapG by binding to the PapD chaperone, thus inhibiting or preventing the formation of the P pili which thereby reduces the capacity of piliated bacteria to attach to host tissues. Similarly, the compounds inhibit the formation of the complex between FimC and FimH, thus inhibiting or preventing the formation of the Type 1. Such interference is particularly effective since the inability of the pilus to attach to target tissues results in the loss of ability of the bacteria to infect the tissue.

Accordingly, the invention is directed generally to compounds of the formula:



wherein each of R_1, R_2 and R_3 is independently a substituted or unsubstituted alkyl (C_{1-10}), substituted or unsubstituted acyl (C_{2-15}), substituted or unsubstituted aryl (C_{6-14}), substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl (C_{7-15}), substituted or unsubstituted heteroarylalkyl or substituted or unsubstituted heterocycloalkyl;R_4 is a carboxy ($-CO_2H$), carboxamide ($-CONH_2$), aldehyde (-CHO), boronate ($-B(OH)_2$), phosphonate ($PO(OH)_2$) or ketone (-COR) wherein R is a halogenated or unsubstituted alkyl (C_{1-3}); and the salts, esters and amines thereof.

The substituents on any alkyl or alkylene moiety may be selected from the group consisting of halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy, carboxamido, cyano, carbonyl, NO_2, $NR'R''$ (wherein each R' and R" is H, alkyl or arylalkyl), alkylthio, alkylthiol, sulfoxide, sulfone, acylamino, amidino, phenyl, benzyl, heteroaryl, phenoxy, benzyloxy, heteroaryloxy, and substituted phenyl, benzyl, heteroaryl, phenoxy, benzyloxy or heteroaryloxy.

Preferably, the active forms of the compounds of the invention are those wherein the chirality of the carbon at R_1 is "S." The same stereochemistry is retained in the analogous compounds and derivatives although the designation of the chirality at each position may be different depending on the nature of the various substitutions made. For example, in an embodiment wherein R_1 is $-CH_2SH$, although the same stereochemistry with regard to the remainder of the molecule remains the same, the chirality would be designated "R." The invention, of course, includes racemic mixtures which include stereoisomers as well as mixtures of the various diasteriomers, as long as this particular form is included.

Included in such derivatives are the salts, especially pharmaceutically acceptable salts.

Salts of carboxylic acids include those derived from inorganic bases such as the sodium, potassium, lithium, ammonium, calcium, magnesium, zinc, aluminum and iron salts and the like, as well as those derived from organic, especially nontoxic, bases such as the primary, secondary and tertiary amines, substituted amines including naturally substituted amines, cyclic amines and basic ion-exchange resins. Examples of such compounds capable of forming salts are isopropyl amine, trimethyl amine, triethyl amine, 2-dimethyl aminoethanol, dicyclohexyl amine, amino acids such as lysine, arginine and histidine, caffeine, procaine, betaene, theobromine, purines, piperazines, and the like.

The compounds of the present invention may also be in esterified form. Typically, the esters are prepared from a hydrocarbyl alcohol. Examples of hydrocarbyl alcohols of C_{1-10} include but are not limited to, cyclopentyl ethyl alcohol, 2-pentyl alcohol, 3-butynyl alcohol, 2,4-dimethyl hexyl alcohol, benzyl alcohol. Particularly preferred are alkyl alcohols. Typical examples of alkyl alcohols include but are not limited to, methyl, ethyl, t-butyl, cyclohexyl. The alkyl esters of the compounds of the invention are particularly preferred, especially alkyl esters wherein the alcohol contains C_{1-4}.

In one embodiment, each R_1, R_2 and R_3 is independently an amino acid residue side chain and R_4 is carboxyl or aldehyde. Preferably, the amino acid residue side chain of each R_1, R_2 and R_3 is independently selected from the group consisting of hydrogen, p-hydroxybenzyl, 2-(3-indolyl)-ethyl, benzyl, 5-imidazole, isopropyl, isobutyl, 2-methylpropyl, methyl and 2-thiomethylethyl and more preferably, R_1 is hydrogen or p-hydroxybenzyl.

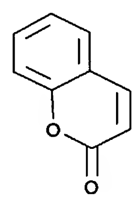
In another embodiment, R_1 is selected from side chains of amino acid residues; R_2 is substituted or unsubstituted alkyl, arylalkyl, heteroarylalkyl, and heterocycloalkyl; R_3 is substituted or unsubstituted alkyl, aryl, and heteroaryl; and R_4 is a carboxyl group (-CO_2H), carboxamide (-$CONH_2$), aldehyde (-CHO), boronate (-$B(OH)_2$) or phosphonate ($PO(OH)_2$). It is preferred that the R_1 is selected from the side chains of hydrophobic aromatic, hydrophobic aliphatic polar and charged amino acid residues, R_2 is substituted or unsubstituted arylalkyl or substituted or unsubstituted heteroarylalkyl, R_3 is substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl, and R_4 is -CO_2H or -CHO.

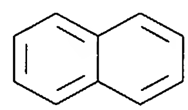
Another preferred embodiment include compounds wherein R_1 is selected from the side chains of hydrophobic aromatic, hydrophobic aliphatic, and polar and charged amino acid residues, R_2 is substituted or unsubstituted arylalkyl or substituted or unsubstituted heteroarylalkyl, R_3 is substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl and R_4 is -CO_2H, -$CONH_2$ or -CHO.

Yet another preferred embodiment includes compounds wherein R_1 is selected from hydrophobic aromatic and hydrophobic aliphatic amino acid residues, R_2 is substituted or unsubstituted arylalkyl or substituted or unsubstituted heteroarylalkyl, R_3 is substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl and R_4 is -

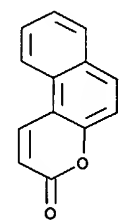
CO_2H. Examples of hydrophobic aromatic or aliphatic amino acid residues are tyrosine, tryptophan, phenylalanine, histidine, valine, leucine, isoleucine, alanine, glycine, cysteine and methionine; examples of polar amino acid residues are serine, threonine, glutamine and asparagine; and examples of charged amino acid residues are lysine, arginine, aspartic acid and glutamic acid. It is particularly preferred that R_4 is $-CO_2H$ as those compounds containing a carboxylic acid functionality demonstrate effective antibacterial properties.

In another embodiment, R_1 is hydrogen, benzyl, 4-aminobutyl and p-hydroxybenzyl; R_2 is $(CH_2)_mA$ wherein m is between 0 and 3 and A is n-butyl, 2-methoxyethyl, benzyl and 2-(3-indolyl)-ethyl; R_3 is isobutyl,



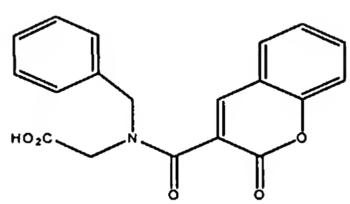


OR

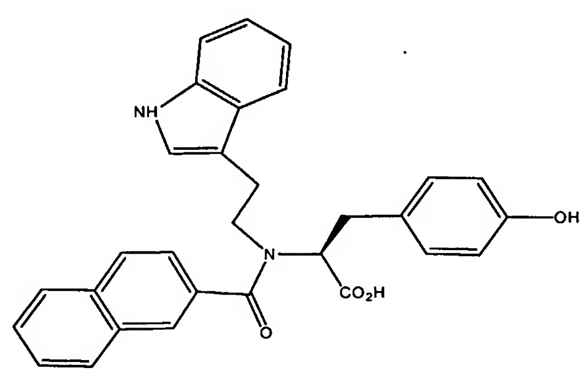


and R_4 is carboxyl.

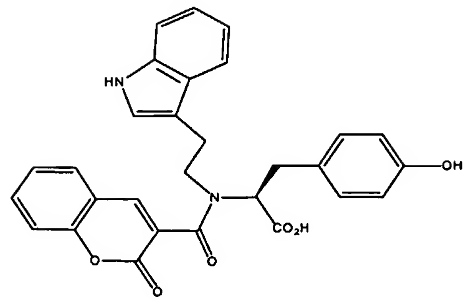
Non-limiting preferred embodiments of the compounds of the present invention include



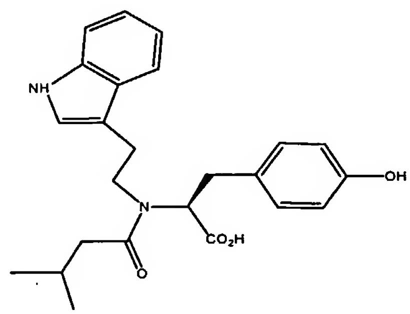
N-Benzyl-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine (Compound 9{*3*, *1*})



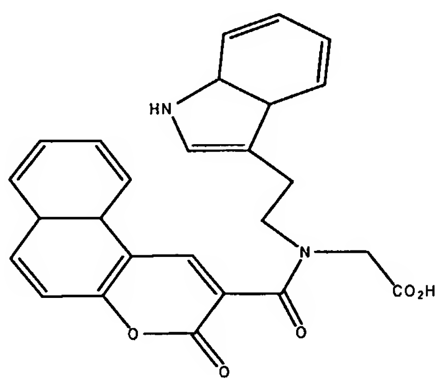
N-[2-(1H-Indol-3-yl)-ethyl]-N-(naphtalene-2-carbonyl)-tyrosine (Compound 19{3, 3})



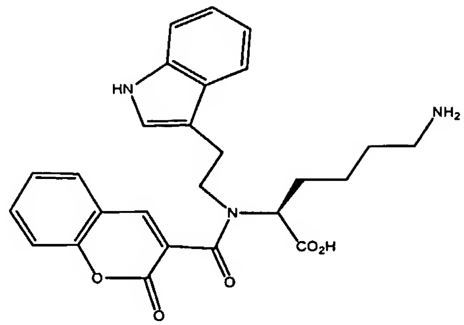
N-[2-(1H-Indol-3-yl)-ethyl]-N-(2-oxo-2H-1-benzopyran-3-carbonyl)-tyrosine
(Compound 17{3, 1})



N-[2-(1H-Indol-3-yl)-ethyl]-N-(3-methyl-butyryl-carbonyl)-tyrosine
(Compound 19{3, 4})

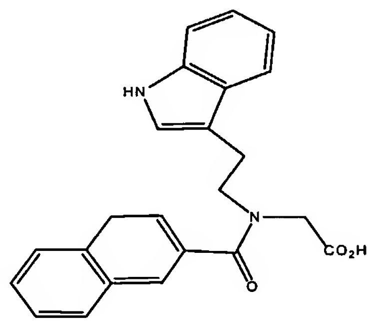


N-[2-(1H-Indol-3-yl)-ethyl]-N-(3-oxo-3H-naphtho[2,1-b]pyran-2-carbonyl)-glycine
(Compound 9{4, 2})



N-[2-(1H-Indol-3-yl)-ethyl]-N-(2-oxo-2H-1-benzopyran-3-carbonyl)-lysine
(Compound 17{4, 1})

and

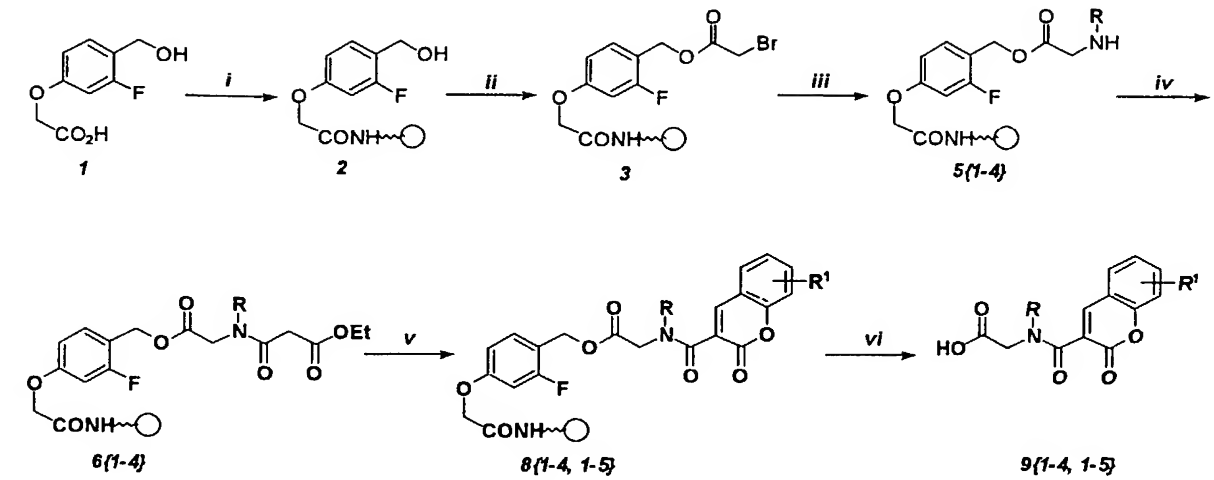


N-[2-(1H-Indol-3-yl)-ethyl]-N-(naphtalene-2-carbonyl)-glycine
(Compound 19{1, 2}).

Synthesis of Antibacterial Compounds

The compounds of the invention can be prepared using approaches illustrated by Reaction Schemes 1-3 and 5. Reaction Schemes 1-3 may be performed in solution or on solid phase. Solid-phase synthesis is employed by first coupling a linker compound to a solid support as illustrated by compound 2 in Reaction Scheme 1. This compound affixed to the solid support is used as a linker in solid phase synthesis as exemplified during the synthesis of compounds 9.

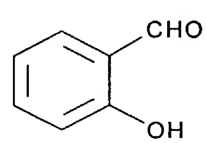
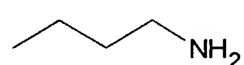
REACTION SCHEME 1

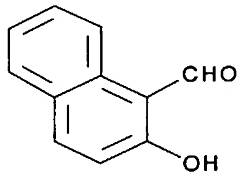
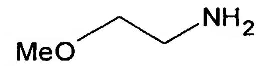
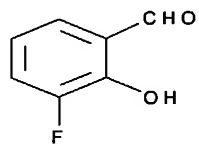
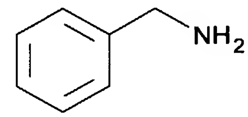
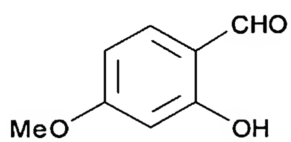
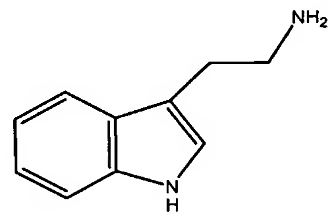
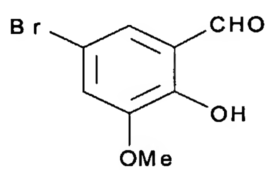


Reaction Scheme 1 reaction conditions: (i) Pentafluorphenol, DIC, EtOAc, TentaGel S NH_2, 0 °C to room temperature; (*ii*) bromoacetic acid, DIC, HOBt, DMAP, THF, room temperature; (*iii*) 4{*1-4*}, CH_3CN, 0 °C; (*iv*) ethyl malonyl chloride, DIPEA, CH_2Cl_2, 0 °C; (*v*) 7{*1-5*}, piperidine, CH_3CN, reflux; (*vi*) aquoeus 1 M LiOH, THF:H_2O:MeOH (3:1:1).

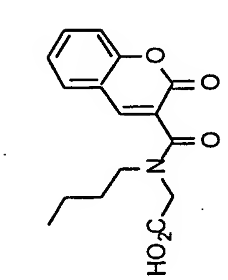
Reaction Scheme 1 is used to synthesize N-substituted glycine derivative compounds, *i.e.*, wherein R_1 is a hydrogen. As is shown in Reaction Scheme 1, acylation of the benzylic alcohol 2 with bromoacetic acid gives 3. Preferably, this acylation reaction is repeated once in order for complete coupling of bromoacetic acid to occur. Nucleophilic substitution of the bromoacetate with an amine in solution gives 5. Amidation of 5 with ethyl malonyl chloride gave a N-alkyl-N-(malonamic acid ethyl ester)-glycine 6. Condensation of 6 with a salicylaldehyde and cleavage of the product from the solid phase under basic conditions gives the N-substituted amino-acid compounds 9. Non-limiting examples of salicyaldehydes and amines which may be used in Reaction Scheme 1 are listed in Table A.

TABLE A

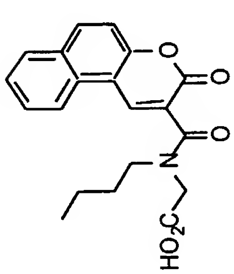
Salicyaldehyde: Reagents 7{1-5}	Amines: Reagents 4{1-4}
7{1} 	4{1} 

7{2} 	4{2} 
7{3} 	4{3} 
7{4} 	4{4} 
7{5} 	

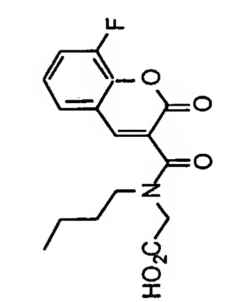
Preferably, Reaction Scheme 1 may be utilized to prepare the library of N-substituted glycine-derivative compounds of Table B.



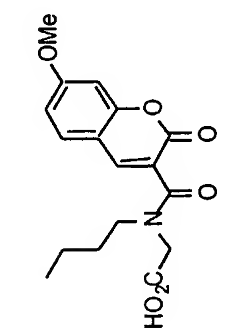
9{1, 1}



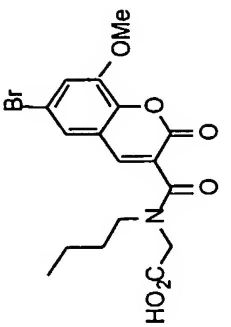
9{1, 2}



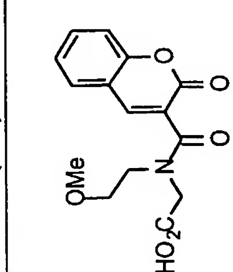
9{1, 3}



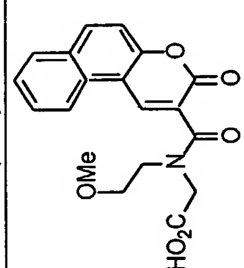
9{1, 4}



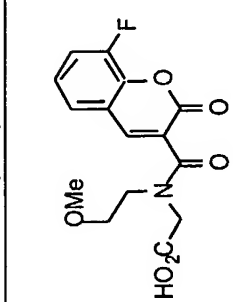
9{1, 5}



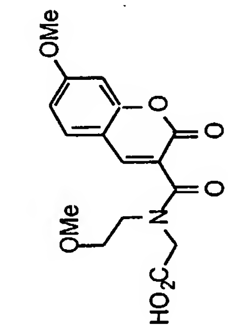
9{2, 1}



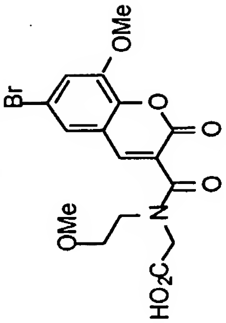
9{2, 2}



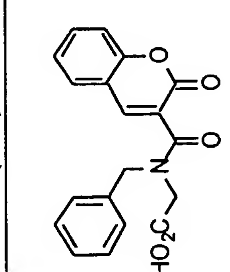
9{2, 3}



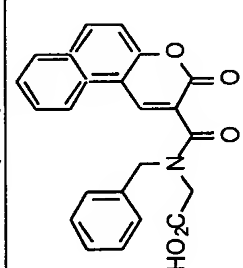
9{2, 4}



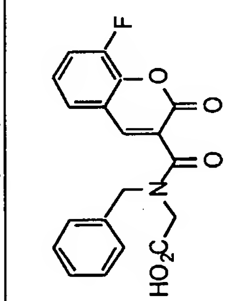
9{2, 5}



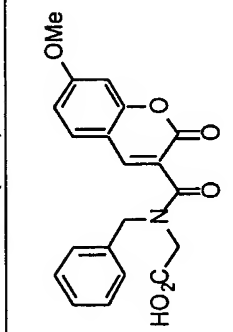
9{3, 1}



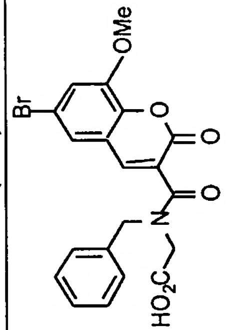
9{3, 2}



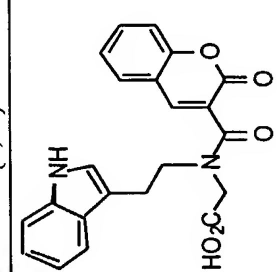
9{3, 3}



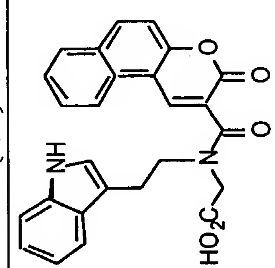
9{3, 4}



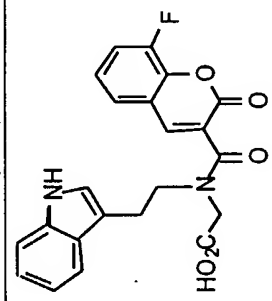
9{3, 5}



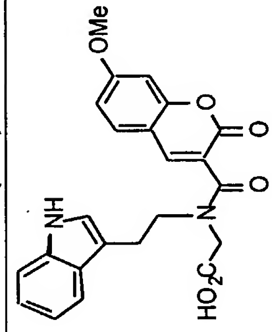
9{4, 1}



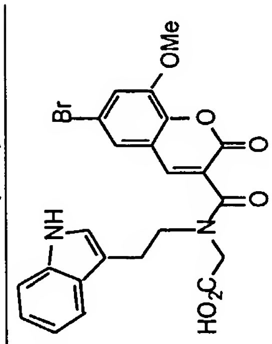
9{4, 2}



9{4, 3}

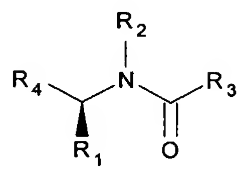


9{4, 4}



9{4, 5}

Preferably, in one embodiment, N-substituted glycine-derivative compounds having the formula:

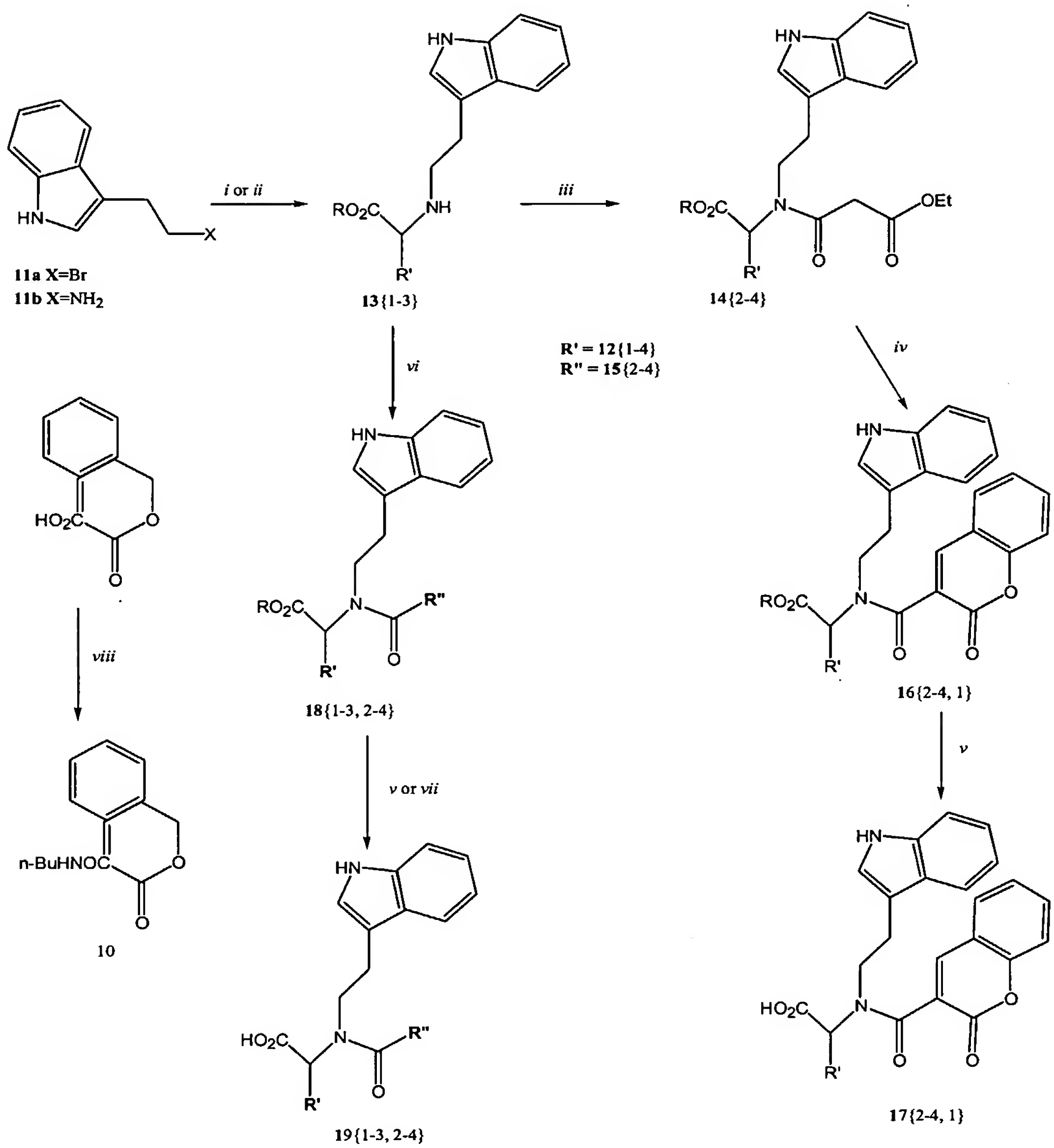


wherein R_1 is hydrogen, R_2 is $(CH_2)_mA$ wherein m is between 0 and 3 and A is selected from the group consisting of n-butyl, benzyl and 3-(2-indolyl)-ethyl, R_3 is coumarin and R_4 is carboxyl, can be synthesized using solid phase synthesis using the fluorinated linker compounds of the present invention as described herein. Preferably, solid phase synthesis of these N-substituted glycine-derivative compounds comprise the steps of: affixing a fluorinated linker compound onto a solid support to give a benzylic alcohol; subjecting the benzylic alcohol to acylation with bromoacetic acid; subjecting the bromoacetate to a nucleophilic substitution with an amine; acylating with ethyl malonyl chloride thereby forming a N-alkyl-N-(malonamic acid ethyl ester)-glycine derivative; condensing the N-alkyl-N-(malonamic acid ethyl ester)-glycine derivative with a salicyaldehyde; and cleaving the compound from the linker compound under acidic or basic conditions.

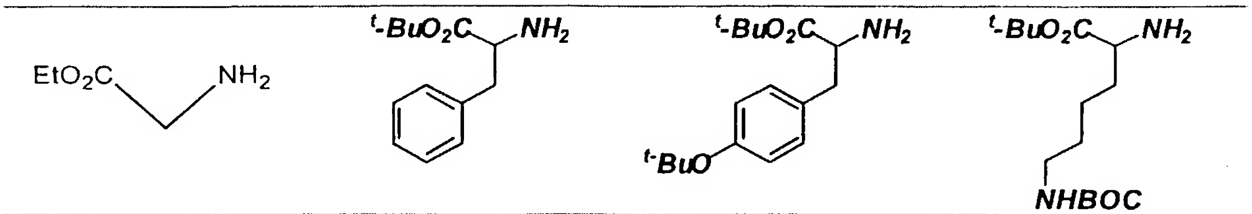
Compounds 6 may also be synthesized in solution by alkylation of amines with ethyl bromoacetate. This is illustrated for the preparation of 13 in Reaction Scheme 2. Subsequent amidation with ethyl malonyl chloride gives 14 in Reaction Scheme 2 which is equivalent to 6 in Reaction Scheme 1.

Further, the N-alkyl-N-(malonamic acid ethyl ester)-amino acid derivative (compound 6 of Reaction Scheme 1) may be prepared in solution as described by Simon et al., Proc. Natl. Sci. USA (1992) 89:9367-9371 and Liskamp et al., Chem Eur. J. (1998) 4:1570-1580, which are both incorporated herein by reference.

REACTION SCHEME 2

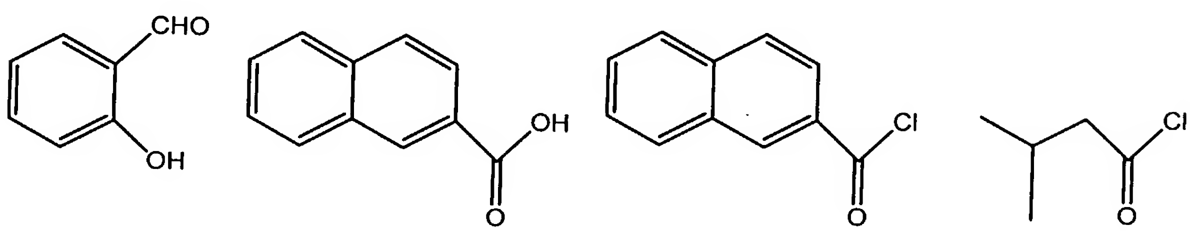


Reaction Scheme 2 reaction conditions: (i) **12**{*1*}, **11b**, ethyl bromoacetate, DMF, 0 °C; (*ii*) **12**{*2-4*}, **11a**, Et_3N, DMF, 70 °C; (*iii*) ethyl malonyl chloride, DIPEA, CH_2Cl_2, 0 °C; (*iv*) **15**{*1*}, piperidine, CH_3CN, reflux; (*v*) for **16**{*2-4, 1*} and **18**{*2-3, 2-4*} TFA:H_2O (2:1), room temperature; (*vi*) for **13**{*1*} pentafluorophenol, DIC, **15**{*2*}, EtOAc; for **13**{*2*} DIC, **15**{*2*}, EtOAc; for **13**{*3*} DIPEA, **15**{*3*}, CH_2Cl_2, 0 °C, for **13**{*3*} DIPEA, **15**{*4*}, CH_2CL_2, 0 °C; (*vii*) for **18**{*1, 2*} aqueous 1 M LiOH, THF:H_2O:MeOH (3:1:1); (*viii*) *n*-butylamine, DIC, CH_2Cl_2, 0 °C.



1	2	3	4

Reagents **12**{*1-4*}.



1	2	3	4

Reagents **15** {1-4}

Additional N-substituted amino acid compounds 17 and 19 wherein R_1 is o-amino, n-butyl, benzyl or p-hydroxybenzyl are prepared using the synthesis of Reaction Scheme 2. As can be seen in Reaction Scheme 2, compound 13 is obtained in solution from a suitable protected amino acid (*e.g.*, Phe, Tyr, Lys) by alkylation with an alkyl halide such as 2-(3-indoyl)-ethyl bromide. Compound 13 is then acylated by a carboxylic acid such as 2-napthalene carboxylic acid, which has been activated *e.g.*, as a pentafluorophenyl ester, with a carbodiamine derivative or an acid chloride. Deprotection of the resulting 18 by treatment with an acid, preferablytrifluoroacetic acid, followed by hydrolysis of the ester moiety then gives 19. In a preferred embodiment, compound 17 is prepared essentially as described above for the synthesis of 9 (Reaction Scheme 1) on solid phase.

Synthesis of Combinational Libraries

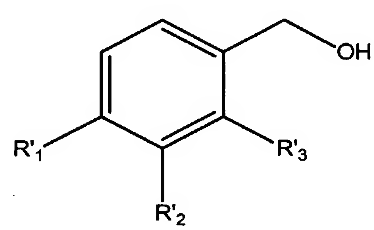
Various compounds similar in structure to the compounds of the present invention may be synthesized using combinational techniques. Suitable combinational techniques include those described in U.S. Patent Nos. 5,736,412, 5,840,500, 5,847,150, 5,852,028, 5,856,107, 5,856,496, 5,859,027 and 5,861,532. These techniques can be performed on solid or solution phase.

The preferred process of the present invention is a "solid phase synthesis" (SPS). Organic synthesis performed on solid phase constitutes an efficient method for preparation of large combinatorial libraries containing structurally distinct molecules. The reaction is

carried out on macroscopic particles made of material insoluble in the reaction medium. A key aspect of any solid-phase synthesis is the choice of a linker compound which will be affixed to the surface of the solid support. The linker compound should be orthogonal to the required reaction conditions and allow quantitative cleavage of the product under mild conditions. Solid supports (e.g., polystyrene resin beads and silica chips) and, concomitantly, solid phase synthesis techniques are routinely utilized in generating combinatorial libraries. Each derivative is usually prepared in sufficient quantity to permit screening and analysis by conventional methods, *e.g.*, HPLC and mass spectral analysis.

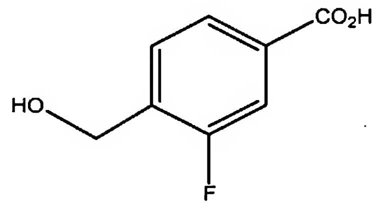
Applicants have shown that ^{19}F chemical shifts, as well as linewidths for compounds attached to polyethylene glycol grafted polystyrene resins (TentaGel resins), approach those in solution. *See* Svensson et al., *Tetahedron Lett.* 37: 7649-7652 (1996). Accordingly, gel-phase ^{19}F NMR spectroscopy is well-suited for adoption of solution-phase chemistry to various solid supports and for optimization of reaction conditions. The insertion of a fluorine atom into a key position on the linker for use in SPS allows the linker to serve as a diagnostic marker during several of the reactions during solid phase synthesis. The fluorine atom of the linker compound allows for the monitoring and optimization of several reactions using ^{19}F NMR spectroscopy such as the attachment of the linker to the solid phase, coupling of the first building block to the linker and cleavage of the product.

Accordingly, the present invention is also directed to a novel class of fluorinated linker compounds having the formula:

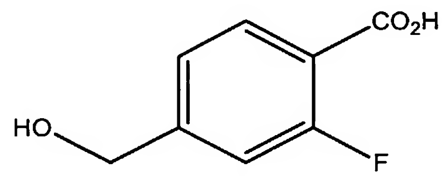


wherein R'_1 is $-CO_2H$, $-(CH_2)_nCO_2H$ or $-O(CH_2)_nCO_2H$ wherein n is between 1 and 10, preferably, n is between 1 and 5, and even more preferably, n is 1 or 2; and R'_2 and R'_3 is independently fluorine or hydrogen provided that when either R'_2 or R'_3 is fluorine, the other is hydrogen.

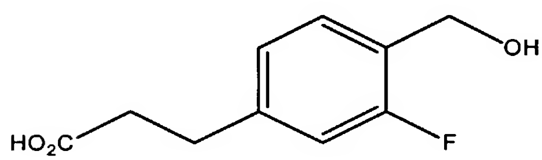
Particularly preferred fluorinated linker compounds include, but are not limited to,



3-fluoro-4-hydroxymethylbenzoic acid (compound 22),

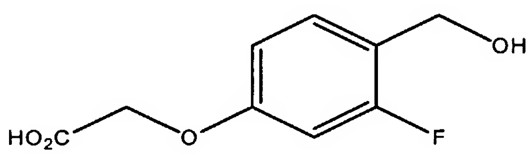


2-fluoro-4-hydroxymethylbenzoic acid (compound 23)



3-fluoro-4-(hydroxymethylphenyl)-propionic acid (compound 29)

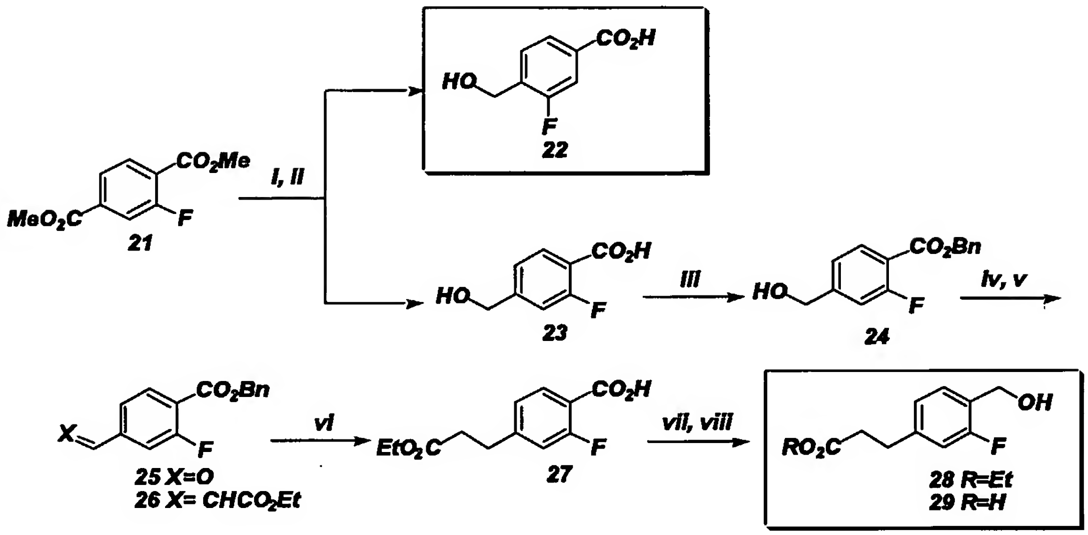
and



3-fluoro-4-hydroxymethyl-phenoxy-acetic acid (compound 1).

These preferred fluorinated linker compounds 1, 22, 23 and 29 are synthesized as shown below in Reaction Schemes 3 and 4.

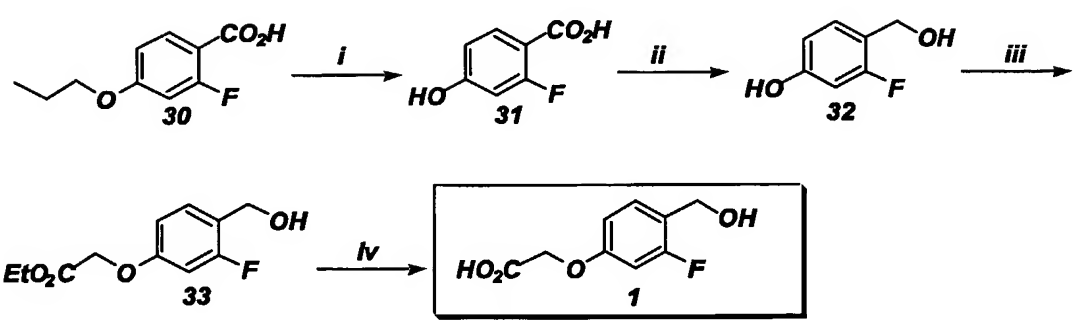
REACTION SCHEME 3



Reaction Scheme 3 reaction conditions: (*i*) aqueous 1 M LiOH, THF:MeOH:H_2O (3:1:1), 0 °C to room temperature; (*ii*) $LiBH_4$, THF, **22** 53%, **23** 40% in two steps; (*iii*) aqueous Cs_2CO_3 (20%), MeOH:H_2O (10:1), then BnBr, DMS, 79%; (*iv*) TPAP (5 mol%), *N*-methylmorpholine *N*-oxide, 4 Å molecular sieves, CH_2Cl_2, 74%; (*v*) NaH, $(EtO)_2P(O)CH_2CO_2C_2H_5$, THF, 0 °C, 76%; (*vi*) Pd/C, H_2, EtOH:EtOAc (3:1) 4 atm, 88%; (*vii*) BH_3-DMS, $(CH_3O)_3B$, THF, 89%; (*viii*) aqueous 1 M LiOH, THF:MeOH:H_2O (3:1:1), 0 °C, 93%.

As shown in Reaction Scheme 3, Linkers 22 and 28 were prepared from dimethyl-2-fluoroterephtalate. Non-selective, basic hydrolysis of one of the ester moieties of dimethyl-2-fluoroterephtalate, followed by reduction of the remaining ester with $LiBH_4$ and chromatographic separation of the two regioisomers gave the fluorinated 4-(hydroxymethyl) benzoic acids, linker compounds 22 and 23. Protection of 23 as a benzyl ester, followed by Swern oxidation and condensation of the resulting aldehyde with triethyl phosphonoacetate afforded 26 which was reduced to 27. Reduction of the carboxyl group of 27 using BH_3-DMS and $(MeO)_3B$ followed by hydrolysis of the ethyl ester then furnished linker 29.

REACTION SCHEME 4



Reaction Scheme 4 reaction conditions: (*i*) BBr_3, CH_2Cl_2, -78 °C to room temperature, 89%; (*ii*) BH_3-DMS, $(CH_3O)_3B$, THF, 90%; (*iii*) $BrCH_2CO_2C_2H_5$, DBU, CH_3CN, reflux, 74%; (*iv*) aqueous 1 M LiOH, THF:MeOH:H_2O (3:1:1) 87%.

Applicants employed 3-fluoro-4-hydroxymethyl-phenoxy-acetic acid, linker 1, to prepare compound 9 {4, 1}, N-[2-(1H-Indol-3-yl)-ethyl]-N-(2-oxo-2H-1-benzopyran-3-carbonyl)-glycine which is a N-substituted glycine derivative exhibiting PapD chaperone inhibiting activity and FimC/FimH inhibiting activity, as indicated in Figure 1.

As shown in Reaction Scheme 4, linker 1 is prepared by BBr_3-induced dealkylation of 2-fluoro-4-propoxybenzoic acid, followed by reduction with BH_3-DMS and $(MeO)_3B$ to give 3-fluoro-4-hydroxymethylphenol 32. *O*-Alkylation of 3-fluoro-4-hydroxymethylphenol 32 with bromoethyl acetate and DBU as a base gives ester and subsequent treatment with LiOH results in linker 1.

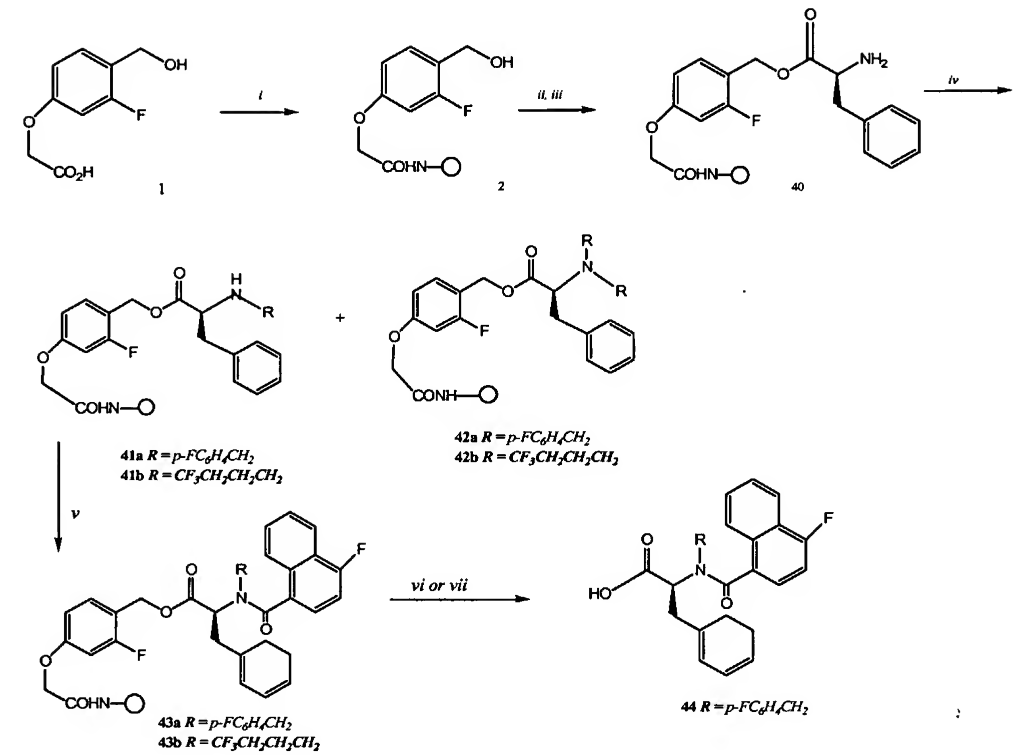
Alternatively, linker 1 may be used to monitor the synthesis of another class of potential pilicides consisting of *N*-alkylated and *N*-acylated amino acids which are different from the glycine derivations previously described. Preferably, such methods for monitoring solid-phase synthesis of such compound include affixing a fluorinated linker compound onto a solid support; utilizing a means for measuring a signal, preferably a ^{19}F resonance, which originates from the linker compound; andutilizing said signal as an internal reference thereby enabling the monitoring of reactions of said solid-phase synthesis of compounds. Preferably, the ^{19}F resonance is measured using ^{19}F NMR spectroscopy.

As shown in Reaction Scheme 5 below, linker 1 is used in combination with gel-phase ^{19}F NMR spectroscopy to develop conditions for solid phase synthesis of another class of potential pilicides consisting of *N*-alkylated and *N*-acylated amino acids which are different from the glycine derivatives produced in Reaction Scheme 1.

Applicants utilized the fluorinated linker 1, in combination with fluorinated building blocks, to establish conditions for reductive alkylation of amino acids that could be applied to both aromatic and aliphatic aldehydes. Phenylalanine resin **40** was prepared by coupling Fmoc-Phe-OH to **2** in the presence of HOBt, DIC and DMAP, followed by removal of the *N*-Fmoc protecting group with 20% piperidine in DMF. The α-amino group in **40** was then alkylated with *p*-fluorobenzaldehyde using $NaBH_3CN$ as reducing agent under different conditions (See Table C of Examples). The ^{19}F resonance originating from the linker moiety of **40** served as internal reference, and integration over the ^{19}F resonance of the *N*-linked *p*-fluorobenzyl residue enabled evaluation of the outcome of the reactions.

Preferably, this method may also be utilized for the reductive alkylation of resin-linked **40** using aliphatic aldehydes. Alkylation of **40** by treatment with 4,4,4-trifluorobutyraldehyde and $NaBH_3CN$ using the conditions of entry 5 in Table C (see Example 5), followed by acylation with 4-fluoronaphtoyl chloride resulted in formation of **43b**. Alternatively, removal of excess aldehyde prior to the addition of $NaBH_3CN$, *i.e.* using the conditions of entry 6 in Table C of Example 5, and subsequent acylation furnished **43b**.

Reaction Scheme 5



Reaction scheme 5 reaction conditions: (*i*) Pentafluorophenol, DIC, TentaGel S NH_2, EtOAc; (*ii*) N^{α}-Fmoc-Phe-OH, HOBt, DIC, DMAP, THF; (*iii)* 20% piperidine in DMF; (*iv*) *p*-F-C_6H_4CHO or $F_3CCH_2CH_2CHO$, MeOH containing 1% HOAc; then $NaBH_3CN$; (*v*) 4-fluoronaphtoyl chloride, DIPEA, CH_2Cl_2, 0 °C to room temperature; (*vi*) aqueous 1M LiOH, THF:MeOH:H_2 O (3:1:1).

Accordingly, solid phase synthesis of a class of potential antibacterial compounds consisting of *N*-alkylated and *N*-acylated amino acids which are different from the glycine derivatives are also synthesized using the fluorinated linker compounds. Preferably, solid phase synthesis of such compounds include the steps of:

a. affixing a fluorinated linker compound 22, 23, 29 or 1 onto a solid support to give a benzylic alcohol;

b. coupling a Fmoc-protected amino acid to the benzylic alcohol thereby producing an amino acid functionalized resin;

c. removing the Fmoc-protecting group from the α-amino group of the product of step (b);

d. alkylating the α-amino group of the product of step (c) by reductive alkyation;

e. removing excess aldehyde from the amino acid functionalized resin;

f. acylating with an acid chloride thereby producing a N-(alkylated)-N-(acylated)-amino acid derivative; and

g. cleaving the compound from the linker under acidic or basic conditions.

Screening Assays

The array of synthesized candidate compounds is screened into relevant assays, *e.g.*, antichaperone or antimicrobial assays, and the compounds are further characterized according to chemical identity and purity using conventional techniques. The assay can be scored on a real-time basis and further modifications made accordingly. Antichaperone binding activity can be measured by any number of direct methods such as monitoring spectral changes in the compound and/or chaperone, determining the extent of compound binding to immobilized chaperone or vice versa, by indirect methods such as competition assays to determine the extent to which these compounds inhibit chaperone binding to target pilus subunits and/or derivatives (Soto, et al., Embo J., (1998) 17:6155; Karlsson et al., Bioorg Med Chem, (1998) 6:2085)) and/or synthetic peptides corresponding to subunit fragments known to bind chaperones (Kuehn, et al., Science, (1993) 262:1234).

Assays to determine the extent of pilus expression in the presence of these compounds may be performed as described in Soto et al., *supra*, and/or by haemagglutination assays as described in Striker et al., Mol Microbiol, (1995) 16:1021.

Assays of inhibition of bacterial binding to target tissues in the presence of these compounds would be performed as described in Striker, et al., *supra*.

Conventional techniques, *e.g.*, radial diffusion method against *E. coli* ML-35P, L. monocytogenes Strain EGD and yeast phase *C. albican*, may be used to evaluate the spectra of the antimicrobial activity for the novel compounds of the present invention.

Antibodies

Antibodies to the compounds of the invention may also be produced using standard immunological techniques for production of polyclonal antisera and, if desired, saving the antibody-producing cells of the immunized host for sources of monoclonal antibody production. Techniques for producing antibodies to any substance of interest are well known. The immunogenicity of the substance may be enhanced by coupling the hapten to a carrier. Carriers useful for this purpose include substances which do not themselves elicit an immune response in the subject mammal. Common carriers used include keyhole limpet hemocyanin (KLH) diptheria taxoid, serum albumin, and the viral coat protein of rotavirus, VP6. Coupling the hapten to the carrier is effected by standard techniques such

as contacting the carrier with the compound in the presence of a dehydrating agent such as dicyclohexylcarbodiimide or through the use of linkers.

The compounds of the invention in immunogenic form are then injected into a suitable mammalian host and antibody titers in the serum are monitored.

Polyclonal antisera may be harvested when titers are sufficiently high. Alternatively, antibody-producing cells of the host such as spleen cells or peripheral blood lymphocytes may be harvested and immortalized. The immortalized cells are then cloned as individual colonies and screened for the production of the desired monoclonal antibodies. The genes encoding monoclonal antibodies secreted by selected hybridomas or other cells may be recovered, manipulated if desired, for example, to provide multiple epitope specificity or to encode a single-chain form and may be engineered for expression in alternative host cells.

Administration of Compounds

The antibacterial compositions of the present invention may be utilized to inhibit pili assembly by providing an effective amount of such compositions to a subject. For use as antimicrobials for treatment of animal subjects, the compounds of the invention can be formulated as pharmaceutical or veterinary compositions. Depending on the subject to be treated, the mode of administration, and the type of treatment desired, *e.g.*, prevention, prophylaxis, therapy; the compounds are formulated in ways consonant with these parameters. A summary of such techniques is found in Remington's Pharmaceutical Sciences, latest edition, Mack Publishing Co., Easton, PA.

For administration to animal or human subjects, the dosage of the compounds of the invention is typically 0.1-100mg/kg. However, dosage levels are highly dependent on the nature of the infection, the condition of the patient, the judgment of the practitioner, and the frequency and mode of administration.

The dosage of such a substance is expected to be the dosage which is normally employed when administering antibacterial drugs to patients or animals, *i.e.* 1 μg - 1000 μg per kilogram of body weight per day. The dosage will depend partly on the route of administration of the substance. If the oral route is employed, the absorption of the substance will be an important factor. A low absorption will have the effect that in the gastro-intestinal tract higher concentrations, and thus higher dosages, will be necessary. Also, the dosage of such a substance when treating infections of the central nervous system (CNS) will be dependent on the permeability of the blood-brain barrier for the substance. As is well-known in the treatment of bacterial meningitis with penicillin, very high dosages are necessary in order to obtain effective concentrations in the CNS.

It will be understood that the appropriate dosage of the substance should suitably be assessed by performing animal model tests, wherein the effective dose level (e.g. ED_{50}) and the toxic dose level (e.g. TD_{50}) as well as the lethal dose level (e.g. LD_{50} or LD_{10}) are established in suitable and acceptable animal models. Dosage levels vary considerably depending on the nature of the infection, the condition of the patient and the frequency and method of administration. Further, if a substance has proven efficient in such animal tests, controlled clinical trials should be performed. Needless to state that such clinical trials should be performed according to the standards of Good Clinical Practice.

In general, for use in treatment, the compounds of the invention may be used alone or in combination with other antibiotics such as erythromycin, tetracycline, macrolides, for example azithromycin and the cephalosporins. Depending on the mode of administration, the compounds will be formulated into suitable compositions to permit facile delivery to the affected areas.

Formulations may be prepared in a manner suitable for systemic administration or topical or local administration. Systemic formulations include those designed for injection (e.g., intramuscular, intravenous or subcutaneous injection) or may be prepared for transdermal, transmucosal, or oral administration. The formulation will generally include a diluent as well as, in some cases, adjuvants, buffers, preservatives and the like. For injection, formulations can be prepared in conventional forms as liquid solutions or suspensions or as solid forms suitable for solution or suspension in liquid prior to injection or as emulsions. Suitable excipients include, for example, water, saline, dextrose, glycerol and the like. Such compositions may also contain amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as, for example, sodium acetate, sorbitan monolaurate, and so forth.

For oral administration, the compounds can be administered also in liposomal compositions or as microemulsions. Suitable dosage forms for oral use include tablets, dispersable powders, granules, capsules, suspensions, syrups, and elixers. Inert diluents and carriers for tablets include, for example, calcium carbonate, sodium carbonate, lactose and talc. Tablets may also contain granulating and disintegrating agents such as starch and alginic acid, binding agents such as starch, gelatin and acacia, and lubricating agents such as magnesium stearate, stearic acid and talc. Tablets may be uncoated or may be coated by unknown techniques; *e.g.*, to delay disintegration and absorption. Inert diluents and carriers which may be used in capsules include, for example, calcium carbonate, calcium phosphate and kaolin. Suspensions, syrups and elixers may contain conventional excipients, for example, methyl cellulose, tragacanth, sodium alginate; wetting agents, such as lecithin and polyoxyethylene stearate; and preservatives, *e.g.* ethyl-p-hydroxybenzoate.

Additionally, various sustained release systems for drugs have also been devised as in U.S. Patent No. 5,624,677, which claims a composition providing a relatively slow release of water-soluble drugs for delivery via the sublingual or buccal routes, for example.

Industrial Applicability

The compounds of the invention are effective in inhibiting a variety of Gram-negative bacteria and have several industrial uses, well known to those skilled in such arts, relating to their antibacterial properties. In general, these uses are carried out by bringing a biocidal or bacterial inhibitory amount of the antibacterial compositions of the present invention into contact with a surface, environment or biozone containing Gram-negative bacteria so that the composition is able to interact with and thereby interfere with the biological function of such bacteria. For example, such antibacterial compositions can be used to prevent or inhibit biofilm formation caused by Gram-negative bacteria. Compositions may be formulated as sprays, solutions, pellets, powders and in other forms of administration well known to those skilled in such arts. For use in these contexts the compounds in the invention may be supplied either as a single compound, in a mixture with several other compounds of the invention or in a mixture with additional antimicrobial agents.

The following examples are intended to illustrate but not limit the invention.

EXAMPLES

Example 1: Synthesis of N-alkylated and N-acylated amino acids in solution.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-N_ε-BOC-lysine *tert*-butyl ester 13{*4*}**. Et_3N (922 μL, 6.61 mmol) was added to a stirred solution of HLys(BOC)O^{t}Bu·HCl (500 mg, 1.63 mmol) and 3-(2-bromoethyl)-indol (1.11 g, 4.96 mmol) in freshly distilled DMF (15 mL). The solution was heated at 70 °C overnight. The solution was cooled to 0 °C and water (15 mL) was added. The water phase was extracted with Et_2O (3x30 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated. Flash chromatography (CH_2Cl_2:MeOH 100:0→99:1→98:2→95:5) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-N_ε-BOC-lysine *tert*-butyl ester (340 mg, 47%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.22 (bs, 1H, C=CHN*H*), 7.61 (d, 1H, *J*=7.3 Hz, Ar-*H*), 7.36 (d, 1H, *J*=8.5 Hz, Ar-*H*), 7.18 (dt, 1H, *J*=7.5, 1.0 Hz, Ar-*H*), 7.11 (dt, 1H, *J*=7.5, 1.0 Hz, Ar-*H*), 7.07 (d, 1H, *J*=2.2 Hz, C=C*H*NH), 4.57 (bs, 1H, N*H*OC(CH_3)$_3$), 3.13 (t, 1H, *J*=8.4 Hz, CH_2C*H*NH)$_3$, 3.08 (m, 2H, C*H*$_2$NHC(O)OC(CH_3)$_3$), 3.00-2.90 and 2.82 (m, 4H, C*H*$_2$C*H*$_2$NH), 1.73 (bs, 1H, N*H*), 1.60-1.50 (m, 2H, C*H*$_2$CHNH), 1.46 (s, 9H, OC(C*H*$_3$)$_3$), 1.39 (s, 9H, OC(C*H*$_3$)$_3$), 1.45-1.28 (m, 4H, C*H*$_2$C*H*$_2$); HRMS FAB$(M+H)^+$ Calcd for $C_{25}H_{40}N_3O_7$ 446.3018, found 446.3012.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(malonamic acid ethyl ester)-N_ε-BOC-lysine *tert*-butyl ester 14{*4*}**. Ethyl malonyl chloride (205 μL, 1.62 mmol) was added to a solution of *N*-[2-

(1H-indol-3-yl)-ethyl]-N_ε-BOC-lysine *tert*-butyl ester (226 mg, 0.54 mmol) and *N*, *N'*-diisopropylethylamine (278 μL, 1.62 mmol) at 0 °C. The reaction was stirred at 0 °C for 60 minutes and then diluted with CH_2Cl_2 (10 mL), washed with aqueous 0.05 M HCl (10 mL), $NaHCO_3$ aq. sat. (10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (heptane:EtOAc, containing 1% HOAc, 2:1→3:2→1:1) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(malonamic acid ethyl ester)-N_ε-BOC-lysine *tert*-butyl ester (275 mg, 97%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.25 (bs, 1H), 7.75 (d, $1H_{min}$, *J*=7.6 Hz, Ar-*H*), 7.57 (d, $1H_{maj}$, *J*=7.9 Hz, Ar-*H*), 7.41 (d, $1H_{maj}$, *J*=8.0 Hz, Ar-*H*), 7.36 (d, $1H_{min}$, *J*=8.0 Hz, Ar-*H*), 7.25-7.05 (m, 3H, Ar-*H*), 4.65 (bs, $1H_{maj}$, N*H*OC(CH_3)$_3$), 4.59 (bs, $1H_{min}$, N*H*OC(CH_3)$_3$),, 4.35-4.06 (m, 4H), 3.75-3.35 (m 4H), 3.09 (m, 4H), 1.50 (s, 9H, OC(CH_3)$_3$), 1.42 (s, 9H, OC(CH_3)$_3$), 1.49-1.43 (m, 3H, OCH_2CH_3), 1.35 (m, 2H); HRMS FAB(M+H)$^+$ Calcd for $C_{30}H_{46}N_3O_7$ 560.3335, found 560.3336.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-N_ε-BOC-lysine *tert*-butyl ester 16**{*4, 1*}. Piperidine (20 μL, 0.20 mmol) was added to a solution of *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(malonamic acid ethyl ester)-N_ε-BOC-lysine *tert*-butyl ester (89 mg, 0.15 mmol) and salicylaldehyde (53 μL, 0.50 mmol) in CH_3CN (12 mL). The solution was heated at reflux over night, cooled to room temperature and diluted with CH_2Cl_2 (15 mL). The organic phase was washed with aqueous 0.05 M HCl (12 mL), $NaHCO_3$ aq. sat. (12 mL) and brine (12 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (heptane:EtOAc, containing 1% HOAc, 3:1→2:1→1:1) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-N_ε-BOC-lysine *tert*-butyl ester (76 mg, 77%). ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.47-7.65 (m, 1H, Ar-*H*), 7.57 (m, 1H, Ar-*H*), 7.45 (m, 1H, Ar-*H*), 7.43-7.45 (m, 2H, Ar-*H*), 7.20 (m, 1H, Ar-*H*), 7.15-7.04 (m, 3H, Ar-*H*), 6.78 (t, 1H, *J*=7.2 Hz, Ar-*H*), 6.56 (t, 1H, *J*=7.4 Hz, Ar-*H*), 4.23 (m, 1H), 3.65 (m, 1H), 3.53 (m, 1H), 3.05 (m, 4H), 2.10 (m, 2H), 1.56 (s, 9H, OC(CH_3)$_3$), 1.46 (m, 4H), 1.40 (s, 9H, OC(CH_3)$_3$); HRMS FAB(M+H)$^+$ Calcd for $C_{35}H_{43}N_3O_7$ 617.3101, found 617.3091.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-lysine 17**{*4, 1*}. TFA:H_2O (2:1, 4 mL) was added to neat *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-N_ε-BOC-lysine *tert*-butyl ester (35 mg, 0.06 mmol). After 60 min at room temperature the solution was concentrated to dryness and the residue was concentrated three times from toluene. Flash chromatography (CH_2Cl_2:MeOH, containing 1% HOAc, 20:1:→8:1→5:1→1:1) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-lysine (17 mg, 64%). ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.20 (bs, $1H_{maj}$, Ar-*H*), 7.83 (bs, $1H_{min}$, Ar-*H*), 7.75 (d, $1H_{maj}$, *J*=7.6 Hz, Ar-*H*), 7.72 (d, $1H_{min}$, *J*=7.9 Hz, Ar-*H*), 7.65 (m, 1H, Ar-*H*), 7.40 (d, $1H_{maj}$, *J*=7.9 Hz, Ar-*H*), 7.35 (m, 1H, Ar-*H*), 7.33

(d, $1H_{min}$, J=8.0 Hz, Ar-H),7.21 (m, 1H, Ar-H), 7.12 (m, 3H, Ar-H), 7.00 (m, 1H, Ar-H), 6.80 (t, $1H_{maj}$, J=7.5 Hz), 6.52 (t, 1H, J=7.4 Hz, Ar-H), 4.04 (bt, $1H_{maj}$, J=7.0 Hz), 3.87 (m. $1H_{min}$), 3.70-3.45 (m, 2H), 3.15-3.0 (m, 2H), 2.97 (t, 1H, J=7.2 Hz), 2.84 (t, 1H, J=7.1 Hz), 2.15 (m, 2H); 1.75 (m, 2H), 1.58 (m, 2H)m 1.33 (m, 2H); HRMS FAB$(M+H)^+$ Calcd for $C_{26}H_{26}N_3O_5Na_2$ 506.1667, found 506.1658.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-phenylalanine *tert*-butyl ester 13**{*2*}. Et_3N (2.01 mL, 14.46 mmol) was added to a stirred solution of HPheO^{t}Bu·HCl (800 mg, 3.61 mmol) and 3-(2-bromoethyl)-indol (2.43 g,10.84 mmol) in freshly distilled DMF (15 mL). The solution was heated at 70 °C over night. The solution was cooled to 0 °C and water (20 mL) was added. The water phase was extracted with Et_2O (3x40 mL), and the combined organic phases were washed with brine (20 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (CH_2Cl_2:MeOH 100:0→400:1→200:1→100:1→20:1) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-phenylalanine *tert*-butyl ester (914 mg, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.24 (s, 1H, C=CHNH), 7.65 (d, 1H, J=7.8 Hz, Ar-H), 7.37 (dd, 1H, J=8.0, 0.8 Hz, Ar-H), 7.32-7.20 (m, 5H, Ar-H), 7.15 (m, 1H, Ar-H), 6.97 (d, 1H, J=2.0 Hz, C=CHNH), 3.54 (1H, t, J=7.1 Hz, CHNH), 3.06-2.87 (m, 6H, -CH_2-), 1.78 (bs, 1H, NH), 1.46 (s, 9H, OC(CH_3)$_3$); HRMS FAB$(M+H)^+$ Calcd for $C_{23}H_{29}N_2O_2$ 365.2229, found 365.2229.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(malonamic acid ethyl ester)-phenylalanine *tert*-butyl ester 14**{*2*}. Ethyl malonyl chloride (260 μL, 2.06 mmol) was added to a solution of *N*-[2-(1*H*-indol-3-yl)-ethyl]-phenylalanine *tert*-butyl ester (250 mg, 0.68 mmol) and *N, N'*-diisopropylethylamine (352 μL, 2.06 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The reaction was stirred at 0 °C for 40 min and then diluted with CH_2Cl_2 (10 mL), washed with aqueous 0.05 M HCl (10 mL), $NaHCO_3$ aq. sat. (10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (heptane:EtOAc, containing 1% HOAc, 3:1→2:1→1:1) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(malonamic acid ethyl ester)-phenylalanine *tert*-butyl ester (304 mg, 93%). ^{1}H NMR ($CDCl_3$,δ 400 MHz) δ 8.26 (bs, 1H, C=CHNH), 7.45 (d, 1H, J=7.8 Hz), 7.36 (m, 1H, Ar-H), 7.32-7.15 (m, 5H, Ar-H), 7.12 (dt, 1H, J=7.4, 1.0 Hz, Ar-H), 6.95 (d, 1H, J=2.2 Hz), 4.21 (q, 2H, OCH_2CH$_3$), 4.12 (m, 1H, CH$_2$CHN), 3.47-3.27 (m 5H, -CH_2-), 3.01-2.85 (m, 2H, -CH_2-), 2.76 (m, 1H, -CH_2-), 1.50 (s, 9H, OC(CH_3)$_3$), 1.44 (m, 3H, OCH$_2$CH_3); HRMS FAB$(M+H)^+$ Calcd for $C_{28}H_{35}N_3O_5$ 479.2545, found 479.2541.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-phenylalanine *tert*-butyl ester 16**{*2, 1*}. Piperidine (43 μL, 0.43 mmol) was added to a solution of *N*-[2-

(1*H*-indol-3-yl)-ethyl]-*N*-(malonamic acid ethyl ester)-phenylalanine *tert*-butyl ester (172 mg, 0.36 mmol) and salicylaldehyde (113 μL, 1.07 mmol) in CH_3CN (15 mL). The solution was heated at reflux over night, cooled to room temperature and diluted with CH_2Cl_2 (20 mL). The organic phase was washed with aqueous 0.05 M HCl (15 mL), $NaHCO_3$ aq. sat. (15 mL) and brine (15 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (heptane:EtOAc, containing 1% HOAc, 3:1→2:1→1:1) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-phenylalanine *tert*-butyl ester (104 mg, 54%). ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.82 (bd, 1H, *J*=7.5 Hz, Ar-*H*) 7.50 (t, 1H, *J*=7.5 Hz, Ar-*H*), 7.39 (m, 3H, Ar-*H*), 7.30 (m, 2H, Ar-*H*), 7.26-7.15 (m, 3H, Ar-*H*), 7.13-7.00 (m, 2H, Ar-*H*), 6.93 (d, 1H, *J*=7.8 Hz, Ar-*H*), 6.86 (bs, 1H, Ar-*H*), 6.80 (t, 1H, *J*=7.5 Hz, Ar-*H*), 6.56 (t, 1H, *J*=7.5 Hz, Ar-*H*), 4.41 (dd, $1H_{min}$, *J*=8.9, 5.8 Hz, $CH_2C\mathit{H}N$), 4.33 (dd, $1H_{maj}$, *J*=8.9, 5.8 Hz, $CH_2C\mathit{H}N$), 3.40 (m, 3H, -$C\mathit{H}_2$-), 2.90-2.75 (m, 3H, -$C\mathit{H}_2$-), 1.55 (s, 9H, $OC(C\mathit{H}_3)_3$); HRMS FAB$(M+H)^+$ Calcd for $C_{33}H_{33}N_2O_5$ 537.2389, found 537.2391.

_N_-[2-(1_H_-Indol-3-yl)-ethyl]-_N_-(2-oxo-2_H_-1-benzopyran-3-carbonyl)-phenylalanine 17{_2, 1_}. TFA:H_2O (2:1, 4 ml) was added to neat *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-phenylalanine *tert*-butyl ester (36 mg, 0.07 mmol). After 90 min at room temperature the solution was concentrated to dryness and the residue was concentrated three times from toluene. Flash chromatography (toluene:EtOAc:HOAc) 85:10:5→80:15:5→60:35:5) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-phenylalanine (24 mg,74%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.58 (bs, $1H_{maj}$, C=C*H*), 8.21 (bs, $1H_{min}$, C=C*H*), 7.40-7.29 (m, 5H, Ar-*H*), 7.24-7.14 (m, 2H, Ar-*H*), 7.05-6.95 (m, 3H, Ar-*H*), 6.85 (bs, 1H, Ar-*H*), 6.79 (bs, 1H, Ar-*H*), 6.72 (t, 1H, *J*=7.5 Hz, Ar-*H*), 6.58 (t, 1H, *J*=7.4 Hz, Ar-*H*), 4.23 (m, 1H, $ArCH_2C\mathit{H}$), 3.56 (m 2H, $NC\mathit{H}_2CH_2$), 3.47 (m, 1H, $ArC\mathit{H}_2CH$), 3.23-3.05 (m, 1H, $ArC\mathit{H}_2CH$), 2.86 (m, 1H, $NCH_2C\mathit{H}_2$), 2.75 (m, 1H, $NCH_2C\mathit{H}_2$); HRMS FAB$(M+H)^+$ Calcd for $C_{29}H_{25}N_2O_5$ 481.1763, found 481,1758.

_N_-[2-(1_H_-Indol-3-yl)-ethyl]-O^{t}Bu-tyrosine _tert_-butyl ester 13{_3_}. Et_3N (888 μL, 6.37 mmol) was added to a stirred solution of HTyr(^{t}Bu)O^{t}Bu·HCl (500 mg, 1.59 mmol) and 3-(2-bromoethyl)-indol (1.07 g, 4.77 mmol) in freshly distilled DMF (15 mL). The solution was heated at 70 °C over night. The solution was cooled to 0 °C and water (15 mL) was added. The water phase was extracted with Et_2O (3x40 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated. Flash chromatography (toluene:EtOH 30:1→10:1→10:2) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-O^{t}Bu-tyrosine *tert*-butyl ester (319 mg, 46%). H NMR ($CDCl_3$, 400 MHz) δ 8.10 (bs, 1H, C=CHN*H*), 7.61 (d, 1H, *J*=7.4 Hz,

Ar-*H*), 7.35 (d, 1H, *J*=8.1 Hz, Ar-*H*), 7.19 (m, 1H, Ar-*H*), 7.13-7.04 (m, 3H, Ar-*H*), 6.98 (d, 1H, *J*=2.2 Hz, C=C*H*), 6.87 (dd, 1H, *J*=6.6, 1.9 Hz, Ar-*H*), 3.45 (dd, 1H, *J*=8.3, 6.3 Hz, $ArCH_2CH$), 3.00-2.75 (m, 6H, $ArCH_2CH$, NCH_2CH_2 and NCH_2CH_2), 1.33 (s, 9H, $OC(CH_3)_3$), 1.28 (s, 9H, $OC(CH_3)_3$); HRMS FAB$(M+H)^+$ Calcd for $C_{27}H_{37}N_2O_3$ 437.2804, found 437.2808.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(malonamic acid ethyl ester)-O^{t}Bu-tyrosine *tert*-butyl ester 14{*3*}**. Ethyl malonyl chloride (181 µL, 0.14 mmol) was added to a solution of *N*-[2-(1*H*-indol-3-yl)-ethyl]-O^{t}Bu-tyrosine *tert*-butyl ester (210 mg, 0.47 mmol) and *N*, *N'*-diisopropylethylamine (246 µL, 0.14 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The reaction was stirred at 0 °C for 50 min and then diluted with CH_2Cl_2 (15 mL), washed with aqueous 0.05 M HCl (15 mL), $NaHCO_3$ aq. sat. (15 mL) and brine (15 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (heptane:EtOAc, containing 1% HOAc, 4:1→3:1→2:1→1:1) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(malonamic acid ethyl ester)-O^{t}Bu-tyrosine *tert*-butyl ester (147 mg, 56%). ^1H NMR ($CDCl_3$, 400 MHz) δ 8.16 (bs, 1H, C=CHN*H*), 7.44 (d, 1H, *J*=7.8 Hz, Ar-*H*), 7.35 (m, 1H, Ar-*H*), 7.19 (m, 1H, Ar-*H*), 7.12 (m, 3H, Ar-*H*), 6.92 (m, 3H, Ar-*H*), 4.38 (t, $1H_{min}$, *J*=7.32 Hz, $ArCH_2CH$), 4.27-4.17 (m, 2H, OCH_2CH_3), 4.06 (dd, $1H_{maj}$, *J*=8.9, 6.3 Hz, $ArCH_2CH$), 3.45-3.25 (m, 5H, $-CH_2-$), 2.96-2.85 (m, 2H, $-CH_2-$), 2.80-2.73 (m, 1H, $-CH_2-$), 1.50 (s, 9H, $OC(CH_3)_3$), 1.26 (m, 12 H, $OC(CH_3)_3$ and OCH_2CH_3); HRMS FAB$(M+H)^+$ Calcd for $C_{32}H_{43}N_2O_6$ 551.3121, found 551.3109.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-O^{t}Bu-tyrosine *tert*-butyl ester 16{*3, 1*}**. Piperidine (26 µL, 0.26 mmol) was added to a solution of *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(malonamic acid ethyl ester)-O^{t}Bu-tyrosine *tert*-butyl ester (121 mg, 0.21 mmol) and salicylaldehyde (69 µL, 0.65 mmol) in CH_3CN (15 mL). The solution was heated at reflux over night, cooled to room temperature and diluted with CH_2Cl_2 (35 mL). The organic phase was washed with aqueous 0.05 M HCl (15 mL), $NaHCO_3$ aq. sat. (15 mL) and brine (15 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (heptane:EtOAc, containing 1% HOAc, 4:1→3:1→2:1→1:1) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-O^{t}Bu-tyrosine *tert*-butyl ester (77 mg, 58%). ^1H NMR ($CDCl_3$, 400 MHz) δ 8.05 (bs, $1H_{min}$, Ar-*H*), 7.98 (bs, $1H_{maj}$ Ar-*H*), 7.60-7.50 (m, 1H, Ar-*H*), 7.37 (m, 1H, Ar-*H*), 7.30-7.10 (m, 7H, Ar-*H*), 6.99 (m, 2H, Ar-*H*), 6.87 (m, 1H, Ar-*H*), 6.80 (dt, 1H, *J*=7.5, 0.9 Hz, Ar-*H*), 4.28 (t, $1H_{min}$, *J*=7.3, $ArCH_2CH$), 4.19 (t, $1H_{maj}$, *J*=7.4, $ArCH_2CH$), 3.50-3.15 (m, 3H, $-CH_2-$), 3.05-2.75 (m, 3H, $-CH_2-$), 1.55 (s, $9H_{maj}$, $OC(CH_3)_3$), 1.44 (s, $9H_{min}$, $O(CH_3)_3$), 1.34 (s, $9H_{min}$, $O(CH_3)_3$), 1.30 (s, $9H_{maj}$, $OC(CH_3)_3$); HRMS FAB$(M+H)^+$ Calcd for $C_{37}H_{40}N_2O_6$ 608.2886, found 608.2878

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-tyrosine 17{*3, 1*}.** TFA:H_2O (2:1, 6 ml) was added to neat *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-O^{t}Bu-tyrosine *tert*-butyl ester (54 mg, 0.08 mmol). After 80 min at room temperature the solution was concentrated to dryness and the residue was concentrated three times from toluene. Flash chromatography (toluene:EtOAc:HOAc) 85:10:5→60:35:5→35:60:5) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-tyrosine (42 mg, 95%). ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.80 (d, 1H_{maj}, *J*=7.9 Hz, Ar-*H*), 7.70 (d, 1H_{min}, Ar-*H*), 7.64 (m, 1H_{min}, Ar-*H*), 7.55 (m, 1$H_{maj,}$ Ar-*H*), 7.30 (m, 1H, Ar-*H*), 7.27 (m, 1H, Ar-*H*), 7.26 (m, 2H, Ar-*H*), 7.19 (m, 1H, Ar-*H*), 7.02 (m, 1H, Ar-*H*), 6.98 (m, 1H, Ar-*H*), 6.78 (m, 2H, Ar-*H*), 6.70 (m, 1H, Ar-*H*), 6.58 (m, 1H, Ar-*H*), 4.32 (bs, 1H, CH_2C*H*N), 3.51 (m, 2H, NC$H_2$$CH_2$), 3.39 (m, 1H, PhC$H_2$CH), 3.35 (m, 1H, PhC$H_2$CH), 2.85 (m, 1H, N$CH_2CH_2$), 2.78 (m, 1H, N$CH_2CH_2$),; ^{13}C NMR (MeOH-$d_4$, 125 MHz) δ 159.7, 157.3, 155.0, 144.0, 137.9, 134.8, 133.8, 132.0, 131.6, 130.5, 130.4, 130.3, 128.3, 126.2, 126.0, 125.2, 124.7, 123.6, 122.4, 120.0, 119.7, 119.6, 119.3, 118.3, 117.6, 117.3, 116.8, 116.5, 112.4, 112.3, 65.2, 52.5, 53.1, 36.5, 35.0, 25.3; HRMS FAB$(M+H)^+$ Calcd for $C_{29}H_{25}N_2O_6$ 497.1712, found 497.1208.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-glycine ethyl ester 13{*1*}.** Ethyl bromoacetate (99 µL, 0.89 mmol) was added to a solution of tryptamine (431 mg, 2.69 mmol) in freshly distilled DMF (5 mL) at 0 °C. The solution was stirred at 0 °C for 1h 45 min and then water (10 mL) was added. The water phase was extracted with Et_2O (3x15mL) and the combined organic phases were dried ($MgSO_4$) and concentrated. Flash chromatography (CH_2Cl_2:MeOH 98:2→96:4) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-glycine ethyl ester (216 mg, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.20 (bs, 1H, C=CHN*H*), 7.63 (d, 1H, *J*=7.8 Hz, Ar-*H*), 7.37 (d, 1H, *J*=8.1, Ar-*H*), 7.19 (dt, 1H, *J*=7.6, 1.1 Hz, Ar-*H*), 7.12 (dt, 1H, *J*= 7.4, 1.0 Hz, Ar-*H*), 7.10 (d, 1H, *J*=2.3 Hz, C=C*H*NH), 4.16 (q, 2H, *J*=7.1 Hz, OC$H_2$$CH_3$), 3.43 (s, 2H, C$H_2$NH); 2 98 (m, 4H, C$H_2CH_2$NH), 1.24 (t, 3H, *J*=7.1 Hz, OCH_2CH_3); HRMS FAB$(M+H)^+$ Calcd for $C_{14}H_{19}N_2O_2$ 247.1446, found 247.1444.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-glycine ethyl ester 18{*1, 2*}.** *N*, *N'*-Diisopropylcarbodiimide (188 µL, 1.21 mmol) was added to a solution of pentafluorophenol (231 mg, 1.25 mmol) in EtOAc (10 mL) at 0 °C. After 30 min 2-naphtoic acid (436 mg, 2.43 mmol) was added and the mixture was stirred for 60 min at 0 °C then *N*-[2-(1*H*-indol-3-yl)-ethyl]-glycine ethyl ester (100 mg, 0.40 mmol) was added. The mixture was stirred over night at room temperature. The precipitate, *N, N'*-diisopropylurea, was removed by filtration and the solvent was diluted with EtOAc (20

mL) and washed with aqueous 0.05 M HCl (2x10 mL), $NaHCO_3$ aq. sat. (2x10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (heptane:EtOAc, containing 1% HOAc, 4:1→3:1→2:1→1:1→1:2) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-glycine ethyl ester (70 mg, 43%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.53 (bs, 1H, C=CHN*H*), 7.89-7.65 (m, 4H, Ar-*H*), 7.56 (m, 2H, Ar-*H*), 7.38 (bd, 1H, *J*=8.1 Hz, Ar-*H*), 7.26 (m, 1H, Ar-*H*), 7.20 (m, 1H, Ar-*H*), 7.07 (m, 1H, Ar-*H*), 6.95 (bd, 1H, *J*=8.1 Hz, Ar-*H*), 6.85 (s, 1H, Ar-*H*), 6.75 (t, 1H, *J*=7.3 Hz, Ar-*H*), 4.36 (s, $2H_{maj}$, (O)CCH_2N), 4.30 (q, $2H_{maj}$, *J*=7.0 Hz, OCH_2CH_3), 4.16 (q, $2H_{min}$, *J*=7.1 Hz, OCH_2CH_3), 3.94 (s, $2H_{min}$, (O)CCH_2N), 3.84 (m, $2H_{min}$, -CH_2-), 3.68 (t, $2H_{min}$, *J*=7.3 Hz, -CH_2-), 3.24 (t, $2H_{min}$, *J*=7.0 Hz, -CH_2-), 3.00 (t, $2H_{maj}$, *J*=7.3 Hz, -CH_2-), 1.35 (t, $3H_{maj}$, *J*=7.1 Hz, OCH_2CH_3), 1.22 (t, $3H_{min}$, *J*=7.1 Hz, OCH_2CH_3).

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-glycine 19**{*1*, *2*}. Aqueous LiOH 1 M (300 μL, 0.29 mmol) was added to a solution of *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-glycine ethyl ester (60 mg, 0.14 mmol) in THF:MeOH:H_2O (3:1:1, 5 mL). After 40 min the solution was cooled to 0 °C and acidified with aqueous 1 M HCl (400 μL). The solution was diluted with EtOAc (10 mL) and a few mL brine added. The organic phase was separated, dried ($MgSO_4$) and concentrated. Flash chromatography (heptane:EtOAc, containing 1% HOAc, 1:1→1:2→1:4) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-glycine (quant. yeild). ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.89 (m, 1H, Ar-*H*), 7.84 (m, 1H, Ar-*H*), 7.76 (d, 1H, *J*=8.4 Hz, Ar-*H*), 7.68 (m, 1H, Ar-*H*), 7.55-7.43 (m, 3H, Ar-*H*), 7.32 (2xd, 1H, *J*=8.1 Hz, Ar-*H*), 7.21 (dd, 1H, *J*=8.4, 1.5 Hz, Ar-*H*), 7.07 (2xt, 1H, *J*=7.4 Hz, Ar-*H*), 6.95 (m, 1H, Ar-*H*), 6.81 (d, 1H, *J*=7.9 Hz, Ar-*H*), 6.54 (dt, 1H, *J*=7.5, 0.8 Hz, Ar-*H*), 4.36 (s, $2H_{maj}$) (O)CCH_2N); 3.89 (t, $2H_{min}$, *J*=7.5 Hz, -CH_2-), 3.82 (s, $2H_{min}$, (O)CCH_2N), 3.67 (t, $2H_{maj}$, *J*=7.0 Hz, -CH_2-), 3.20 (t, $2H_{min}$, *J*=7.3 Hz, -CH_2-), 3.00 (t, $2H_{maj}$, *J*=6.8 Hz, -CH_2-); HRMS FAB$(M+H)^+$ Calcd for $C_{23}H_{21}N_2O_3$ 373.1552, found 373.1546.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-phenylalanine *tert*-butyl ester 18**{*2*, *2*}. *N*, *N*′-Diisopropylcarbodiimide (40 μL, 0.26 mmol) was added to a solution of *N*-[2-(1*H*-indol-3-yl)-ethyl]-phenylalanine *tert*-butyl ester (50 mg, 0.13 mmol), 2-naphtoic acid (49 mg, 0.27 mmol) and 1-hydroxybenzotriazole (56 mg, 0.41 mmol) in THF (5 mL) at 0 °C. The mixture was stirred over night at room temperature and then concentrated. The residue was diluted with EtOAc (5 ml), the precipitate, *N*, *N*′-diisopropylurea, was removed by filtration and the solvent was diluted with EtOAc (20 mL). The organic phase was washed with aqueous 0.05 M HCl (2x10 mL), $NaHCO_3$ aq. sat. (2x10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (heptane:EtOAc,

containing 1% HOAc, 4:1) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-phenylalanine *tert*-butyl ester (21 mg, 30%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (s, 1H, Ar-*H*), 7.96-7.69 (m, 4H, Ar-*H*), 7.55 (m, 2H, Ar-*H*), 7.39 (m, 3H, Ar-*H*), 7.26 (m, 3H, Ar-*H*), 7.18 (m, 2H, Ar-*H*), 6.98 (m, 1H, Ar-*H*), 6.65 (m, 1H, Ar-*H*), 6.55 (m, 1H, Ar-*H*), 4.61 (m, $1H_{min}$), 4.30 (m, $1H_{maj}$), 3.94 (m, $1H_{min}$), 3.71 (m, $1H_{maj}$), 3.65-3.40 (m, 2H) 3.25-3.09 (m, 1H), 2.98 (m, 1H), 2.70 (m, 1H), 1.62 (s, $9H_{maj}$, $OC(CH_3)_3$), 1.58 (s, $9H_{min}$, $OC(CH_3)_3$); HRMS FAB$(M+H)^+$ Calcd for $C_{34}H_{35}N_2O_3$ 519.2647, found 519.2638.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-phenylalanine 19**{*2*, *2*}. TFA:H_2O (2:1, 6 mL) was added to neat *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-phenylalanine *tert*-butyl ester (16 mg, 0.003 mmol). After 1.5 h at room temperature the solution was concentrated to dryness, and then concentrated three times from toluene. Flash chromatography (toluene:EtOAc:HOAc 90:8:2→85:10:5→60:35:5) gave *N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-phenylalanine (10 mg, 70%). ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.86 (m, 2H, Ar-*H*), 7.81 (m, 2H, Ar-*H*), 7.67 (s, 1H, Ar-*H*), 7.54 (m, 3H, Ar-*H*), 7.38 (m, 4H, Ar-*H*), 7.30-7.05 (m, 8H, Ar-*H*), 6.87 (d, 1H, *J*=6.9 Hz, Ar-*H*), 6.81 (dt, 1H, *J*=7.4, 1.1 Hz, Ar-*H*), 6.74 (s, 1H, Ar-*H*), 6.35 (m, 1H, Ar-*H*), 4.69 (dd, $1H_{min}$, *J*=10.4, 3.7 Hz, CH_2CHN), 4.60 (dd, $1H_{maj}$, *J*=10.9, 5.1 Hz, CH_2CHN), 3.60 (dd, 1H, *J*=13.7, 10.8 Hz, -CH_2-), 3.50 (dd, 1H, *J*=14.0, 5.0 Hz, -CH_2-), 3.39 (m, 1H, -CH_2-), 2.95 (m, 1H, -CH_2-), 2.68 (m, 2H, -CH_2-); HRMS FAB$(M+H)^+$ Calcd for $C_{30}H_{27}N_2O_3$ 463.2021, found 463.2010.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-O^{t}Bu-tyrosine *tert*-butyl ester 18**{*3*, *3*}. 2-Naphtoylchloride (18 mg, 0.09 mmol) in CH_2Cl_2 (1 mL) was added to an ice cooled solution of *N*-[2-(1*H*-indol-3-yl)-ethyl]-O^{t}Bu-tyrosine *tert*-butyl ester (14 mg, 0.03 mmol) and DIPEA (16 μL, 0.09 mmol) in CH_2Cl_2 (5 mL). The solution was stirred at 0 °C for 1h 15 min, allowed to reach room temperature and then diluted with CH_2Cl_2 (10 mL). The organic phase was washed with aqueous 0.05 M HCl, $NaHCO_3$ aq. sat. and brine (10 mL each), dried ($MgSO_4$) and concentrated. Flash chromatorgraphy (heptane:EtOAc, containing 1% HOAc, 4:1→1:1) gave *N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-O^{t}Bu-tyrosine *tert*-butyl ester (14 mg, 74%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (bs, 1H), 7.95-7.73 (m, 5H, Ar-*H*), 7.55 (m, 2H, Ar-*H*), 7.40 (bd, 1H, *J*=7.9 Hz, Ar-*H*), 7.19 (m, 3H, Ar-*H*), 7.00 (bd, 1H, *J*=7.8 Hz, Ar-*H*), 6.85 (m, 1H, Ar-*H*), 6.63 (m, 1H, Ar-*H*), 4.60 (m, $1H_{min}$, CH_2CHN), 4.27 (m, $1H_{maj}$, CH_2CHN), 3.93 (m, 1H, -CH_2-), 3.71 (m, 1H, -CH_2-), 3.58 (m, 1H, -CH_2-), 3.43 (m, 2H, -CH_2-), 3.20 (m, 1H, -CH_2-), 3.00 (m, 2H, -CH_2-), 2.67 (m, 2H, -CH_2-), 1.60 (m, 9H, $OC(CH_3)_3$), 1.31 (m, 9H, $OC(CH_3)_3$).

N-[2-(1H-Indol-3-yl)-ethyl]-N-(naphtalene-2-carbonyl)-tyrosine 19{*3, 3*}.
TFA:H_2O (2:1, 5 mL) was added to neat *N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-O^{t}Bu-tyrosine *tert*-butyl ester (14 mg, 0.02 mmol). The solution was stirred at room temperature for 1h and 30 min, concentrated to dryness, and then concentrated three times from toluene. Flash chromatography (toluene:EtOAc:HOAc, 90:8:2→85:10:5→60:35:5) gave *N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-tyrosine (10 mg, 87%). ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.85-7.65 (m, 4H, Ar-*H*), 7.54 (m, 2H, Ar-*H*), 7.35-7.05 (m, 6H, Ar-*H*), 6.85-6.54 (m, 4H, Ar-*H*), 6.40 (m, 1H, Ar-*H*), 4.56 (m, $1H_{maj+min}$, CH_2CHN), 4.00-2.60 (m, 6H, -CH_2-).

2-Oxo-2*H*-1-benzopyran-3-carboxylic acid butylamide (10).
N, N'-Diisopropylcarbodiimide (81 µL, 0.52 mmol) was added to a solution of coumarine-3-carboxylic acid (100 mg, 0.52 mmol) and *n*-butylamine (104 µL, 1.05 mmol) in CH_2Cl_2 (5 mL) and the solution was stirred at room temperature over night. The precipitate was removed by filtration, the solvent diluted with CH_2Cl_2 (25 mL) and washed with aqueous 0.05 M HCl (2x10 mL), $NaHCO_3$ aq.sat. (2x10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated. Flash chromatography(heptane:EtOAc, containing 1% HOAc, 5:1) gave 2-oxo-2*H*-1-benzopyran-3-carboxylic acid butylamide (26 mg, 20%). ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.80 (s, 1H, C=C*H*), 7.80 (m, 1H, Ar-*H*), 7.71 (m, 1H, Ar-*H*), 7.41 (m, 1H, Ar-*H*), 3.41 (t, 2H, *J*=7.0 Hz, CH_2CH_2N), 1.60 (m, 2H, -CH_2-), 1.43 (m, 2H, -CH_2-), 0.96 (t, 2H, *J*=7.3 Hz, CH_2CH_3); HRMS FAB$(M+H)^+$ Calcd for $C_{14}H_{16}NO_3$ 24.1130, found 246.1133.

Example 2: Synthesis of Fluorinated Linkers

General. TLC was performed on Silica Gel 60 F_{254} (Merck). Flash column chromatography employed Grace Amicon silica gel 60 Å (30-60 µm) with distilled solvents. CH_2Cl_2 and CH_3CN were distilled from calcium hydride immediately before use; THF was distilled from sodium-benzophenone ketyl and ethanol was dried over 4 Å molecular sieves. Reactions in these solvents were performed under an atmosphere of nitrogen; solvents, reactant solutions and liquid reagents being transferred via oven-dried syringes. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-400 spectrometer for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.27 ppm) and $CDCl_3$ (δ_C 77.23 ppm) as internal standard] and MeOH-d_4 [residual CH_2DOD (δ_H 3.31 ppm) and CD_3OD (δ_C 49.00 ppm) as internal standard] at 295 K. High-resolution mass spectra [HRMS (EI+)] were recorded on a Jeol JMS-SX 102 spectrometer.

3-Fluoro-4-hydroxymethylbenzoic acid (22) and 2-fluoro-4-hydroxymethylbenzoic acid (23). Aqueous LiOH (1 M, 1.55 mL, 1.55 mmol) was added to a stirred solution of dimethyl 2-fluoroterephthalate (300 mg, 1.41 mmol) in THF:MeOH:H_2O (3:1:1, 4 mL) at 0 °C. The resultant solution was allowed to reach room temperature and was then stirred over night. The solution was diluted with EtOAc (10 mL), the water phase was separated and cooled to 0 °C, acidified with aqueous 1 M HCl and extracted with EtOAc (4x20 mL). The combined organic phases were dried (Na_2SO_4), and concentrated. Flash chromatography (heptane:EtOAc 7:1→6:1, containing 1% HOAc) gave a mixture of terephtalic acid mono-methyl esters (215 mg, 77%), as well as terephtalic acid (70 mg). The mixture of terephtalic acid mono-methyl esters (80 mg, 0.494 mmol) was dissolved in THF (0.8 mL) and added to a stirred solution of $LiBH_4$ (28 mg, 1.21 mmol) in THF (0.8 mL). The solution was stirred at ambient temperature for 30 min, then ethanol (3.2 mL) was added droppwise, and the resultant slurry was stirred over night. The reaction mixture was cooled to 0 °C and acetone (0.2 mL) followed by aqueous 1 M HCl (1.2 mL) were added to give a clear solution. The water phase was extracted with EtOAc (4x10 mL) and the combined organic phases were washed once with brine (10 mL), dried (Na_2SO_4) and concentrated. Flash chromatography (heptane:EtOAc 4:1→1:1, containing 1% HOAc) of the residue gave **22** (36 mg, 53%) and **23** (28 mg, 40%). Compound **22** had ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.94 (dd, 1H, *J*=7.9, 1.4 Hz, Ar-*H*), 7.64 (dd, 1H, *J*=10.6, 1.4 Hz, Ar-*H*), 7.58 (t, 1H, *J*=7.6 Hz, Ar-*H*), 4.73 (s, 2H, ArCH_2OH); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 168.6, 162.6, 160.1, 135.1, 130.2, 126.8, 117.2, 58.7; HRMS (EI+) Calcd for $C_8H_7O_3F$ 170.0379, found 170.0374. Compound **23** had ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.90 (t, 1H, *J*=7.9, 1.4 Hz, Ar-*H*), 7.21 (m, 2H, Ar-*H*), 4.65 (s, 2H, ArCH_2OH); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 167.5, 165.05, 162.5, 151.5, 133.5, 122.9, 115.7, 64.0; HRMS (EI+) Calcd for $C_8H_7O_3F$ 170.0379, found 170.0374.

Benzyl 2-fluoro-4-hydroxymethylbenzoate (24). A solution of **23** (492 mg, 2.89 mmol) in MeOH:H_2O (10:1, 13.2 mL) was titrated to pH 7 with aqueous Cs_2CO_3 (20%, ~3.5 mL). The resultant solution was concentrated to dryness and the residue was concentrated twice from freshly distilled DMF (2x8 mL). Benzyl bromide (0.411 mL, 3.47 mmol) was added to a slurry of the solid cesiumsalt in DMF (8 mL), and the reaction solution was stirred over night at ambient temperature. The mixture was concentrated and water was added to the residue. The water phase was extracted with EtOAc (4x50 mL) and the combined organic phases were dried (Na_2SO_4) and concentrated. Flash chromatography (heptane:EtOAc 4:1→1:1) of the residue gave **24** (588 mg, 79%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (t, 1H, *J*=7.8 Hz, Ar-*H*), 7.45 (m, 2H, Ar-*H*), 7.38 (m, 3H, Ar-*H*), 7.17 (m, 2H, Ar-*H*), 5.35 (s, 2H, PhCH_2O), 4.76 (d, 2H, *J*=6 Hz, ArCH_2OH); ^{13}C NMR ($CDCl_3$,

400 MHz) δ 164.3, 164.2, 163.8, 161.2, 148.9, 148.8, 135.9, 132.5, 128.8, 128.4, 128.3, 121.8, 121.7, 117.6, 117.5, 115.0, 114.8, 67.1, 64.1; HRMS (EI+) Calcd for $C_{15}H_{13}O_3F$ 260.0848, found 260.0846.

Benzyl 4-((*E*)-2-ethoxycarbonyl-vinyl)-2-fluorobenzoate (26). Tetrapropylammonium perruthenate (TPAP, 5 mg, 0.013 mmol, 5 mol%) was added in one portion to a stirred slurry of **24** (70 mg, 0.269 mmol), 4-methylmorpholine *N*-oxide (47 mg, 0.403 mmol) and 4 Å molecular sieves (134 mg) in CH_2Cl_2 (5 mL). The resultant slurry was stirred at ambient temperature for 30 min and then filtrated through a pad of silica gel eluted with CH_2Cl_2 (50 mL). Concentration of the solution and flash chromatography (heptane:EtOAc 7:1) of the residue gave the aldehyde **25** (51 mg, 74%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.05 (d, 1H, *J*=1.5 Hz, ArC*H*O), 8.12 (t, 1H, *J*=7.3 Hz, Ar-*H*), 7.74 (dd, 1H, *J*=7.9, 1.3 Hz, Ar-*H*), 7.64 (dd, 1H, *J*=10.1, 1.3 Hz, Ar-*H*), 7.48-7.33 (m, 4H, Ar-*H*), 5.45 (s, 2H ArCH_2O); HRMS (EI+) Calcd for $C_{15}H_{11}O_3F$ 258.0692, found 258.0693. $(EtO)_2P(O)CH_2CO_2C_2H_5$ (397 mg, 1.77 mmol) was added to a slurry of NaH (55-65%, 62 mg, 1.55 mmol) in THF (5mL) at 0 °C. After a few minutes the mixture became clear and was allowed to reach room temperature after which **25** (286 mg, 1.10 mmol) dissolved in THF (5 mL) was added. After stirring the mixture for 1h, H_2O (20 mL) was added and the solution was poured into Et_2O (30 mL). The water phase was extracted with Et_2O (4x30 mL) and the combined organic layers were washed with H_2O (15 mL) and brine (2x10mL). The organic phase was dried ($MgSO_4$), concentrated and the residue was purified by flash chromatography (heptane:EtOAc 8:1) to give **26** (276 mg, 76%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (t, 1H, *J*=7.7 Hz, Ar-*H*), 7.62 (d, 1H, *J*=16.0 Hz, Ar-*H*), 7.46 (m, 2H, Ar-*H*), 7.41-7.35 (m, 4H, Ar-*H*), 7.27 (m, 1H, ArC*H*=CH), 6.49 (d, 1H, *J*=16.0 Hz, CH=C*H*CO$_2$Et), 5.38 (s, 2H PhCH_2O), 4.28 (q, 2H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 1.34 (t, 3H, *J*=7.1 Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.1, 163.6, 160.8, 141.7, 140.9, 135.6, 132.7, 128.6, 128.3, 128.1, 123.4, 119.6, 116.0, 115.8, 67.1, 60.9, 14.2; HRMS (EI+) Calcd for $C_{19}H_{17}O_4F$ 328.1110, found 328.1111.

4-(2-Ethoxycarbonylethyl)-2-fluorobenzoic acid (27). A small amount of 10% Pd-C was added to a solution of **26** (254 mg; 0.774 mmol) in dry EtOH:EtOAc (3:1, 8 mL) and the resultant mixture was hydrogenated at 4 atm for 15 h. The mixture was filtered through a pad of Celite, concentrated and the residue was purified by flash chromatography (heptane:EtOAc 3:1→1:1, containing 1% HOAc) to give **27** (164 mg, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.94 (bs, 1H, CO$_2$*H*), 7.93 (bs, 1H, Ar-*H*), 7.05 (d, 1H, *J*=7.7, Ar-*H*), 7.01 (d, 1H, *J*=11.4 Hz, Ar-*H*), 4.15 (q, 2H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 3.00 (t, 2H, *J*=7.6 Hz, ArCH_2CH$_2$), 2.65 (t, 2H, *J*=7.6 Hz, ArCH$_2$CH_2), 1.24 (t, 3H, *J*=7.1 Hz, $CO_2CH_2CH_3$);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5, 161.8, 159.4, 142.7, 129.5, 124. 1, 115.3, 60.5, 59.3, 35.5, 30.4, 14.1; HRMS (EI+) Calcd for $C_{12}H_{13}O_4F$) 240.0798, found 240.0801.

Ethyl 3-(3-fluoro-4-hydroxymethylphenyl)-propionate (28). BH_3-DMS (92 mg, 1.21 mmol) was added to a solution of **27** (145 mg, 0.604 mmol) and $(CH_3O)_3B$ (251 mg, 2.41 mmol) in THF (8 mL) was added. The resultant mixture was stirred at ambient temperature until **27** was consumed (TLC) and a clear solution was obtained. MeOH (1 mL) was added and the mixture was concentrated to dryness. The residue was dissolved in Et_2O (10 mL) and washed with water (5 mL) and $NaHCO_3$ (2x3 mL). The organic solution was dried ($MgSO_4$), concentrated and the residue was purified by flash chromatography (heptane:EtOAc 4:1) to give **28** (106 mg, 89%). ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32 (t, 1H, *J*=7.8 Hz, Ar-*H*), 6.99 (dd, 1H, *J*=7.7, 1.6 Hz, Ar-*H*), 6.91 (dd, 1H, *J*=11.0, 1.5 Hz, Ar-*H*), 4.72 (d, 2H, *J*=6.0 Hz, $ArCH_2OH$), 4.12 (q, 2H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 2.93 (t, 2H, *J*=7.7 Hz, $ArCH_2CH_2$), 2.60 (t, 2H, *J*=7.7 Hz, $ArCH_2CH_2$), 1.80 (bt, 1H, *J*=6.0 Hz, $ArCH_2OH$), 1.23 (t, 3H, *J*=7.1 Hz,

$CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5, 162.3, 159.8, 143.1, 129.9, 124.6, 115.7, 60.9, 59.7, 35.9, 30.8, 14.6; HRMS (EI+) Calcd for $C_{12}H_{15}O_3F$ 226.1005, found 260.1002.

3-(3-Fluoro-4-hydroxymethylphenyl)-propionic acid (29). Aqueous LiOH (1 M, 0.448 mL, 0.448 mmol) was added to a stirred solution of **28** (84.5 mg, 0.373 mmol) in THF:MeOH:H_2O (3:1:1, 1.5 mL) at 0 °C. After 30 min the solution was allowed to reach room temperature and was then stirred for 4.5 h. The solution was then recooled to 0 °C, acidified with aqueous 1 M HCl and poured into EtOAc:H_2O (8:2, 10 mL). The water phase was extracted with EtOAc (2x5 mL) and the combined organic phases were washed once with brine (6 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (heptane:EtOAc 4:1→3:1, containing 1% HOAc) to give **29** (69 mg, 93%). ^1H NMR (MeOH-d_4, 400 MHz) δ 7.35 (t, 1H, J=7.8 Hz, Ar-H), 7.04 (dd, 1H, J=7.8, 1.5 Hz, Ar-H), 6.96 (dd, 1H, J=11.2, 1.5 Hz, Ar-H), 4.62 (s, 2H ArCH_2OH), 2.91 (t, 2H, J=7.5 Hz, ArCH_2CH_2), 2.60 (t, 2H, J=7.5 Hz, ArCH_2CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.4, 162.0, 159.6, 143.2, 143.1, 129.6, 129.2, 129.1, 126.1, 124.2, 124.1, 115.0, 114.8, 109.0, 57.7, 35.3, 30.4; HRMS (EI+) Calcd for $C_{10}H_{11}O_3F$ 198.0692, found 198.0692.

2-Fluoro-4-hydroxybenzoic acid (31). 2-Fluoro-4-propoxybenzoic acid (**30**, 1.21 g, 6.09 mmol) was dissolved in CH_2Cl_2 (16 mL), the solution was cooled to -78 °C and BBr_3 (1 M in CH_2Cl_2, 18.3 mL, 18.3 mmol) was added. The solution was slowly allowed to reach room temperature and was then stirred at ambient temperature over night. H_2O (35 mL) was added and the resultant mixture was poured into Et_2O (20 mL). The water phase was extracted with Et_2O (4x40 mL) and the combined organic phases were dried ($MgSO_4$). Concentration and flash chromatography (heptane:EtOAc 2:1→1:2, containing 1% HOAc) of the residue gave **31** (850 mg, 89%). ^1H NMR (MeOH-d_4, 400 MHz) δ 7.80 (t, 1H, J=8.7 Hz, Ar-H), 6.54 (dd, 1H, J=8.7, 2.3 Hz, Ar-H), 6.54 (dd, 1H, J=12.9, 2.3 Hz, Ar-H); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 167.7, 166.7, 165.1, 164.1, 135.0, 112.6, 104.6; HRMS (EI+) Calcd for $C_7H_5O_3F$ 156.0222, found 156.0221.

3-Fluoro-4-hydroxymethylphenol (32). Compound **31** (679 mg, 4.35 mmol) in THF (20 mL) was added to a stirred solution of $(CH_3O)_3B$ (3.62 g, 34.8 mmol) and BH_3-DMS (1.32 g, 17.4 mmol) in THF (50 mL). The mixture was stirred at ambient temperature until **31** was consumed (TLC) and a clear solution was obtained. MeOH (26 mL) was added, the mixture was concentrated, and the residue was co-concentrated from MeOH (3x50 mL). The residue was flash chromatographed (heptane:EtOAc 2:1→1:1) to give **32** (558 mg, 90%). ^1H NMR (MeOH-d_4, 400 MHz) δ 7.21 (t, 1H, J=8.6 Hz, Ar-H), 6.57 (dd, 1H, J=8.4,

2.4 Hz, Ar-*H*), 6.49 (dd, 1H, *J*=11.8, 2.4 Hz, Ar-*H*), 4.53 (s, 2H, ArCH_2OH); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 164.1, 161.7, 160.1, 131.9, 120.1, 112.3, 103.5, 58.8; HRMS (EI+) Calcd for $C_7H_7O_2F$ 142.0430, found 142.0427.

Ethyl (3-fluoro-4-hydroxymethylphenoxy)-acetate (33). DBU (777 mg, 5.10 mmol) was added to a stirred solution of **32** (558 mg, 3.93 mmol) and ethyl a-bromoacetate (1.18 g, 7.07 mmol) in CH_3CN (50 mL). The solution was refluxed over night and then cooled to room temperature. The solution was poured into Et_2O (100 mL) and washed with aqueous 0.05 M HCl (2x50 mL) and brine (40 mL), dried (Na_2SO_4) and concentrated. The residue was flash chromatographed (toluene:EtOAc 6:1→3:1→1:1) to give **33** (108 mg, 74%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31 (t, 1H, *J*=8.5 Hz, Ar-*H*), 6.68 (dd, 1H, *J*=8.3, 2.8 Hz, Ar-*H*), 6.63 (dd, 1H, *J*=11.5, 2.5 Hz, Ar-*H*), 4.66 (s, 2H, ArCH_2OH), 4.60 (s, 2H, OCH_2CO_2H), 4.27 (q, 2H, *J*=7.1 Hz, OCH_2CH_3), 1.98 (bs, 1H, O*H*), 1.30 (t, 3H, *J*=7.1 Hz, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.7, 162.6, 160.1, 158.9, 130.5, 121.3, 110.3, 102.9, 65.7, 61.7, 59.1, 14.3; HRMS (EI+) Calcd for $C_{11}H_{13}O_4F$ 228.0797, found 228.0795.

3-Fluoro-4-(hydroxymethyl-phenoxy)-acetic acid (1). Aqueous LiOH (1 M, 1.03 mL, 1.03 mmol) was added to a stirred solution of **33** (157 mg, 0.691 mmol) in THF:MeOH:H_2O (3:1:1, 10 mL) at 0 °C. After 30 min the solution was allowed to reach room temperature and was then stirred at ambient temperature for 1.5 h. The solution was recooled to 0 °C, acidified with aqueous 1 M HCl and poured into EtOAc (30 mL). The water phase was extracted with EtOAc (3x10 mL) and the combined organic phases were washed with brine (15 mL), dried (Na_2SO_4) and concentrated. The residue was flash chromatographed (heptane:EtOAc 1:2, containing 1% HOAc) to give **1** (119 mg, 87%). ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.34 (t, 1H, *J*=8.6 Hz, Ar-*H*), 6.76 (ddd, 1H, *J*=8.3, 2.5, 0.8 Hz, Ar-*H*), 6.70 (dd, 1H, *J*=11.8, 2.5 Hz, Ar-*H*), 4.66 (s, 2H, ArCH_2OH), 4.58 (s 2H, OCH_2OH); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 172.4, 163.8, 161.4, 160.4, 131.6, 122.5, 111.4, 103.5, 66.2, 58.6; HRMS (EI+) Calcd for $C_9H_9O_4F$ 200.0484, found 200.0486.

<u>Example 3: Use of Fluorinated Linkers to Monitor Solid-Phase Synthesis</u>

Applicants have previously shown that ^{19}F chemical shifts, as well as linewidths for compounds attached to polyethylene glycol grafted polystyrene resins (TentaGel resins), approach those in solution. This makes gel-phase ^{19}F NMR spectroscopy well suited for adoption of solution-phase chemistry to various solid supports and for optimization of reaction conditions. Linker 1 was now attached to an amino

functionalized TentaGel resin (TentaGel S NH_2), and to amino functionalized polystyrene, after which the primary hydroxyl group of the linker was acylated with *p*-fluorobenzoyl chloride. ^{19}F NMR spectroscopy of the two functionalized resins revealed that the ^{19}F line widths were 2-3 times larger for the polystyrene resin, as compared to the TentaGel resin, when $CDCl_3$, DMSO-d_6, benzene-d_6, or pyridine-d_5 were used to swell the resins. This result is in good agreement with previous studies of the influence of the resin on the quality of ^1H NMR spectra of solid-supported compounds.

As shown in Reaction Scheme 1, attachment of **1** to a TentaGel S NH_2 resin using 1-hydroxy-7-azabenzotriazole (HOAt) and *N,N'*-diisopropylcarbodiimide (DIC) for activation, was also found to result in some coupling of the activated linker to the hydroxyl group of **2**. This was indicated in the ^{19}F NMR spectrum which showed a peak for acylated linker at d -115 ppm in addition to the peak at d -117 ppm which originates from linker-resin **2** (peak ratio 1:3). Employing milder reaction conditions, *i.e.* coupling of the linker activated as the pentafluorophenyl ester, circumvented this *O*-acylation (Figure 1a), but still allowed complete coupling of **1** to the resin as revealed by monitoring with bromophenol blue. Acylation of **2** with bromoacetic acid (3 eq.) in the presence of 1-hydroxybenzotriazole (HOBt), DIC and a catalytic amount of *N,N'*-dimethylaminopyridine (DMAP), did not give complete conversion into **3** (Figure 1b). As judged by ^{19}F NMR spectroscopy, the conversion of **2** into **3** was improved from 90% to 100% by repeating the acylation using 1.5 eq. of bromoacetic acid (Figure 1c). It should be pointed out that high quality spectra were obtained within minutes for samples of resin (~ 100 mg) in an ordinary NMR tube using a standard NMR spectrometer.

Nucleophilic substitution of the α-brominated ester **3** with *n*-butylamine, followed by amidation of **5** with ethyl malonyl chloride and *N,N'*-diisopropylethylamine (DIPEA), did not induce any change in the ^{19}F NMR chemical shift. The ^{19}F NMR spectrum of **8** showed that Knoevenagel condensation of **6** with salicylaldehyde in CH_3CN using piperidine as base, was accompanied by a side-reaction (cf Figure 1d). An additional peak having a shift identical to that of **2** indicated that some cleavage (~20%) of **9** from the solid support had occurred. Different reaction conditions were explored, but did not lead to any improvement. Pilicide **9** was finally cleaved from the resin using either aqueous LiOH in THF:MeOH:H_2O or the optimized conditions based on TFA as described above. In both cases **9** was isolated in 48% yield based on the overall capacity of the resin.

Example 4: Solid-Phase Synthesis of N-Alkylated and N-Acylated Glycine Derivatives

Methods. TLC was performed on Silica Gel 60 F_{254} (Merck). Flash column

chromatography employed Grace Amicon silica gel 60 Å (30-60 μm) with distilled solvents. CH_2Cl_2, DMF and CH_3CN were distilled from calcium hydride immediately before use, and THF were distilled from sodium-benzophenone ketyl. Reactions in these solvents were performed under an atmosphere of nitrogen; solvents, reactant solutions and liquid reagents being transferred via oven-dried syringes. Solid phase synthesis of **9**{*1-4, 1-5*} was performed on TentaGel S NH_2 resin (130 μm, 0.224 mmol/g). ^1H and ^{13}C NMR spectra were obtained on a Bruker DRX-400 spectrometer for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.27) and $CDCl_3$ (δ_C 77.23) as internal standard] and MeOH-d_4 [residual CHD_2OD (δ_H 3.31) and CD_3OD (δ_C 49.00) as internal standard] at 295 K. Proton resonances were assigned from COSY experiments. The peaks were assigned from mixtures of rotamers. Gel-phase ^{19}F NMR spectra were recorded with a Bruker ARX-400 spectrometer for solutions in $CDCl_3$, MeOH-d_4 or DMSO-d_6 with CCl_3F (δ_F 0.0 ppm) as internal standard. Positive ion fast-atom bombardment [HRMS, FAB$(M+H)^+$] and electron impact mass spectroscopy [HRMS, (EI+)] were recorded on a Jeol JMS-SX 102 spectrometer.

Attachment of the linker (3-fluoro-4-hydroxymethylphenoxy)-acetic acid. *N, N′*-Diisopropylcarbodiimide (DIC, 835 μL , 5.40 mmol) was added to an ice-cold solution of pentafluorophenol (1.99 g, 10.80 mmol) in EtOAc (30 mL). After 30 min (3-fluoro-4-hydroxymethylphenoxy)-acetic acid (1.13 g, 5.67 mmol) was added and the solution was stirred at 0 °C for 60 min. The mixture was then added to the resin (10 g, 2.70 mmol, pre-swollen in EtOAc) and the mixture was agitated at ambient temperature for 12 h. The resin was washed with EtOAc (100 mL), MeOH (80 mL), THF (80 mL) and dry THF (40 mL) and dried under vacuum.

General procedure for preparation of Compounds **9**{*1-4, 1-5*}:

Resin-bound bromoacetic acid (3). DIC (1.04 mL, 6.75 mmol) was added to a solution of bromoacetic acid (1.13 g, 8.10 mmol) and 1-hydroxybenzotriazole (HOBt, 729 mg, 5.40 mmol) in THF (30 mL) and the solution was stirred at ambient temperature for 60 min. The mixture and a catalytic amount of *N, N′*-dimethylaminopyridine (DMAP, 108 mg, 0.89 mmol) in THF (10 mL) were then added to **2** (2.70 mmol, pre-swollen in dry THF) and the mixture was agitated over night. The resin was washed with THF, MeOH, THF (each 100 mL) of and dry THF (20 mL) and dried under vacuum. In order to obtain complete coupling of bromoacetic acid to **2** the reaction was repeated once.

Resin-bound *N*-alkyl-glycine 5{*1-4*}. A solution of each amine **4**{*1-4*} (3 eq, 8.10 mmol) in freshly distilled CH_3CN (30 mL), for tryptamine freshly distilled DMF was used as solvent, was added to **3** (2.70 mmol, pre-swollen in dry CH_3CN or DMF) at 0 °C. The resin was

agitated at 0 °C for 90 min and then washed with CH_3CN, MeOH, THF (100 mL each) and dry THF (20 mL) before being dried under vacuum.

Resin-bound *N*-alkyl-*N*-(malonamic acid ethyl ester)-glycine 6{*1-4*}. Ethyl malonyl chloride (1.02 mL, 8.10 mmol) dissolved in CH_2Cl_2 (10 mL) was added to a suspension of **5**{*1-4*} (2.7 mmol) and *N,N'*-diisopropylethylamine (DIPEA, 1.38 mL, 8.10 mmol) in CH_2Cl_2 (20 mL) at 0 °C and the resin was agitated at 0 °C for 60 min. The resin was washed with CH_2Cl_2, MeOH, THF (100 mL each) and dry THF (20 mL), and then dried under vacuum.

Resin-bound 8{*1-4, 1-5*}. Each amine of the resins **6**{*1-4*} was split into five portions (each approximately 1.9 g resin, 0.54 mmol) which were reacted with the five different salicylaldehydes **7**{*1-5*}. A solution of each salicyaldehyde (3 eq., 1.54 mmol) in freshly distilled CH_3CN (7 mL) was added to **6**{*1-4*} which had been pre-swollen in CH_3CN. The mixture was heated to reflux as piperidine (1.2 eq., 61 μL, 0.62 mmol) in CH_3CN (1 mL) was added. After refluxing over night the resin allowed to reach room temperature before being washed with CH_3CN, MeOH, THF (each 50 mL) and dry THF (10 mL). Then the resin was dried under vacuum.

***N*-alkyl-*N*-(2-oxo-*2H*-1-benzopyran-3-carbonyl)-glycine 9{*1-4, 1-5*}**.
Aqueous LiOH (1 M, 5 mL, 5 mmol) was added to **8**{*1-4, 1-5*} (approximately 0.54 mmol) in THF:H_2O:MeOH (3:1:1, 40 mL) at 0 °C. After 2.5 h at ambient temperature the resin was filtered of and washed with THF, acetic acid and THF (each 50 mL). The filtrate was concentrated almost to dryness and then concentrated from toluene (2x50 mL). The residue was dissolved in a mixture of EtOAc (30 mL) and aqueous HCl (0.05 M, 10 mL), the organic layer was separated and washed with aqueous HCl (0.05 M, 2x10 mL). The organic phase was dried (Na_2SO_4) and concentrated. The crude products were purified by flash column chromatography as stated below for each compound.

***N*-Butyl-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9{*1, 1*}**. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 55% yield; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.97 (s, $1H_{maj+min}$, C=C*H*), 7.60 (m, $2H_{maj+min}$, Ar-*H*), 7.36 (m, $2H_{maj+min}$, Ar-*H*), 4.26 (s, $2H_{maj}$, (O)CCH_2N), 4.06 (s, $2H_{min}$, (O)CCH_2N), 3.35 (t, $2H_{min}$, *J*=7.4 Hz, NCH_2CH_2), 3.33 (t, $2H_{maj}$, *J*=7.7 Hz, NCH_2CH_2), 1.61 (m, $2H_{maj+min}$, NCH_2CH_2), 1.41 (m, $2H_{min}$, CH_2CH_3), 1.26 (m, $2H_{maj}$, CH_2CH_3), 0.96 (t, $3H_{min}$, *J*=7.3 Hz, CH_2CH_3), 0.85 (t, $3H_{maj}$, *J*=7.3 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.8, 172.5, 166.4, 166.0, 158.5, 158.1, 154.3, 144.2, 143.5, 133.3, 133.2, 129.2, 129.1, 125.3, 125.2, 124.7, 124.5, 118.4, 118.3, 117.1,

116.9, 50.7, 50.1, 47.5, 47.1, 30.3, 29.2, 20.2, 19.9, 13.9, 13.8; HRMS (EI+) Calcd for $C_{16}H_{17}NO_5$ 303.1106, found 303.1104.

***N*-Butyl-*N*-(3-oxo-3*H*-naphtho[2,1-*b*]pyran-2-carbonyl)-glycine 9{*1*, *2*}**. Flash column chromatography with toluene:EtOAc:HOAc 70:25:5; 30% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.82 (s, 1H_{maj}, C=C*H*), 8.80 (s, 1H_{min}, C=C*H*), 8.29 (d, 1H_{maj}, *J*=8.4 Hz, Ar-*H*), 8.18 (d, 1H_{min}, *J*=8.1 Hz, Ar-*H*), 8.04 (d, 1H_{maj}, *J*=9.1 Hz, Ar-*H*), 7.97 (d, 1H_{min}, *J*=8.9 Hz, Ar-*H*), 7.91 (d, 1H_{maj}, *J*=8.1 Hz, Ar-*H*), 7.84 (d, 1H_{min}, *J*=7.8 Hz, Ar-*H*), 7.71 (bt, 1H_{maj}, *J*=7.4 Hz, Ar-*H*), 7.60 (m, 2$H_{maj+min}$, Ar-*H*), 7.45 (d, 1H_{maj}, *J*=8.9 Hz, Ar-*H*), 7.40 (d, 1H_{min}, *J*=8.9 Hz, Ar-*H*), 6.95 (bs, 1$H_{maj+min}$, CO_2*H*), 4.26 (s, 2H_{maj}, (O)CH_2N), 4.09 (s, 2H_{min}, (O)CH_2N), 3.55 (bt, 2H_{min}, *J*=7.6 Hz, NCH_2CH_2), 3.34 (bt, 2H_{maj}, *J*=7.7 Hz, NCH_2CH_2), 1.61 (m, 2$H_{maj+min}$, NCH_2CH_2), 1.38 (m, 2H_{min}, CH_2CH_3), 1.22 (m, 2H_{maj}, CH_2CH_3), 0.94 (t, 3H_{min}, *J*=7.3 Hz, CH_2CH_3), 0.82 (t, 3H_{maj}, *J*=7.3 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.3, 166.9, 166.5, 158.5, 158.2, 154.4, 140.8, 140.1, 134.8, 134.7, 130.5, 130.4, 129.3, 129.2, 129.0, 128.9, 126.7, 126.6, 123.2, 123.0, 121.9, 116.8, 116.7, 112.9, 112.8, 50.7, 47.6, 47.3, 30.3, 29.2, 20.2, 19.9, 14.0, 13.8; HRMS (EI+) Calcd for $C_{20}H_{19}NO_5$ 353.1263, found 353.1263.

***N*-Butyl-*N*-(8-fluoro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9{*1*, *3*}**. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 19% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 s (1$H_{maj+min}$, C=C*H*), 7 38 (m, 2H, Ar-*H*), 7.30 (m, 2H, Ar-*H*), 4.24 (s, 2H_{maj}, (O)CCH_2N), 4.06 (s, 2H_{min}(O)CCH_2N), 3.55 (t, 2H_{min}, *J*=7.6 Hz, NCH_2CH_2), 3.32 (t, 2H_{maj}, *J*=7.8 Hz, NCH_2CH_2), 1.60 (m, 2$H_{maj+min}$, NCH_2CH_2), 1.40 (m, 2H_{min}, CH_2CH_3), 1.26 (m, 2H_{maj}, CH_2CH_3), 0.96 (t, 3H_{min}, *J*=7.3 Hz, CH_2CH_3), 0.85 (t, 3H_{maj}, *J*=7.3 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1, 165.9, 156.7, 142.9, 142.8, 125.5, 125.2, 125.1, 124.1, 124.0, 120.1, 119.6, 119.4, 50.7, 47.2, 30.3, 29.2, 20.2, 20.0, 14.0, 13.8; HRMS (EI+) Calcd for $C_{16}H_{16}NO_5F$ 321.1012, found 321.1014.

***N*-Butyl-*N*-(7-methoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9{*1*, *4*}**. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5; 22% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.93 (s, 1$H_{maj+min}$, C=C*H*), 7.48 (m, 2$H_{maj+min}$, Ar-*H*), 6.91 (dd, 1$H_{maj+min}$, *J*=8.7, 2.3 Hz, Ar-*H*), 6.85 (m, 2$H_{maj+min}$, Ar-*H*), 4.23 (bs, 2H_{maj}, (O)CCH_2N), 4.05 (bs, 2H_{min}, (O)CCH_2N), 3.91 (s, 3H_{min}, OCH_3), 3.83 (s, 3H_{min}, OCH_3), 3.53 (bt, 2H_{min}, *J*=7.2 Hz, NCH_2CH_2), 3.33 (t, 2H_{maj}, *J*=7.7Hz, NCH_2CH_2), 1.59 (m, 2$H_{maj+min}$, NCH_2CH_2), 1.38 (m, 2H_{min}, CH_2CH_3), 1.23 (m, 2H_{maj}, CH_2CH_3), 0.94 (t, 3H_{min}, *J*=7.3 Hz, CH_2CH_3), 0.84 (t, 3H_{maj}, *J*=7.3 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5, 166.9, 166.5, 164.1, 164.0, 158.8, 158.5, 156.3, 144.7, 144.0, 130.2, 130.0, 120.7, 120.5, 113.6, 112.1, 111.9, 100.8, 100.7, 56.1, 50.7, 47.6, 47.1, 30.3, 29.1, 22.8, 20.2, 19.9, 14.3, 14.0, 13.8; HRMS (EI+) Calcd for $C_{17}H_{19}NO_6$ 333.1212, found

333.1212.

***N*-(6-Bromo-8-methoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl)-*N*-butyl-glycine 9{*1*, *5*}.** Flash column chromatography with heptane:EtOAc containing 1% HOAc 3:1→2:1, 45% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.85 (s, 1$H_{maj+min}$, C=C*H*), 7.29-7.15 (m, 2$H_{maj+min}$, Ar-*H*), 4.21 (bs, 2H_{maj}, (O)CCH_2N), 4.01 (bs, 2H_{min}, (O)CCH_2N), 3.96 (s, 3H_{maj}, OCH_3), 3.93 (s, 3H_{min}, OCH_3), 3.50 (bt, 2H_{min}, *J*=7.5 Hz, NCH_2CH$_2$), 3.28 (bt, 2H_{maj}, *J*=7.7 Hz, NCH_2CH$_2$), 1.57 (m, 2$H_{maj+min}$, NCH$_2$CH_2), 1.40-1.15 (m, 2$H_{maj+min}$, CH_2CH$_3$), 0.93 (bt, 3H_{min}, *J*=7.3 Hz, CH$_2$CH_3), 0.83 (bt, 3H_{maj}, *J*=7.3 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4, 172.3, 165.9, 165.8, 157.3, 157.0, 147.9, 147.8, 142.9, 142.3, 129.2, 128.3, 125.9, 125.7, 125.5, 125.4, 122.3, 122.1, 120.0, 119.8, 117.9, 117.8, 117.5, 117.4, 56.7, 50.6, 47.2, 32.0, 30.0, 29.1, 29.1, 22.8, 20.1, 19.9, 14.3, 14.0, 13.8; HRMS (EI+) Calcd for $C_{17}H_{18}NO_6Br$ 411.0317, found 411.0315.

***N*-(2-Methoxy-ethyl)-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9{*2*, *1*}.** Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 40% yield; ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.12 (s, 1H_{maj}, C=C*H*), 8.04 (s, 1H_{min}, C=C*H*), 7.69 (m, 2$H_{maj+min}$, Ar-*H*), 7.39 (m, 2$H_{maj+min}$, Ar-*H*), 4.32 (s, 2H_{maj}, (O)CH_2N), 4.19 (s, 2H_{min}, (O)CH_2N), 3.74 (t, 1H_{min}, *J*=5.2 Hz, NCH_2CH$_2$), 3.62 (t, 1H_{min}, *J*=5.2 Hz, NCH$_2$CH_2), 3.57 (m, 1H_{maj}, NCH_2CH$_2$), 3.53 (m, 1H_{maj}, NCH$_2$CH_2), 3.34 (s, 3H_{min}, OCH_3), 3.30 (s, 3H_{maj}, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.9, 166.5, 166.4, 158.4, 158.3, 154.2, 144.3, 144.1, 133.3, 133.2, 129.1, 128.9, 125.3, 125.2, 124.5, 124.4, 118.4, 118.3, 117.0, 116.9, 71.4, 71.3, 59.0, 58.8, 52.0, 50.4, 48.6, 47.7; HRMS FAB$(M+H)^+$ Calcd for $C_{15}H_{16}NO_6$ 306.0977, found 306.0979

***N*-(2-Methoxy-ethyl)-*N*-(3-oxo-3*H*-naphtho[2,1-*b*]pyran-2-carbonyl)-glycine 9{*2*, *2*}.** Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 35% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.83 (s, 1H_{maj}, C=C*H*), 8.77 (s, 1H_{min}, C=C*H*), 8.22 (m, 1H, Ar-*H*), 7.96 (t, 1H, *J*=9.8 Hz, Ar-*H*), 7.85 (m, 1H, Ar-*H*), 7.63 (m, 1H, Ar-*H*), 7.55 (m, 1H, Ar-*H*), 7.37 (m, 1H, Ar-*H*), 4.33 (bs, 2H_{maj}, (O)CH_2N), 4.20 (bs, 2H_{min}, (O)CH$_2$N), 3.80 (m, 1H_{min}, NCH$_2$CH$_2$), 3.67 (m, 1H_{min}, NCH$_2$CH$_2$), 3.54 (bs, 2H_{maj}, NCH$_2$CH$_2$ and NCH$_2$CH$_2$), 3.32 (s, 3H_{min}, OCH$_3$), 3.27 (s, 3H_{maj}, OCH$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 167.0, 158.6, 154.3, 140.5, 134.6, 130.4, 129.3, 129.2, 129.1, 129.0, 128.4, 126.7, 123.1, 122.7, 121.9, 116.7, 112.9, 109.3, 71.2, 70.9, 59.0, 58.8, 50.4, 48.8, 47.7; HRMS FAB$(M+H)^+$ Calcd for $C_{19}H_{18}NO_6$ 356.1134, found 356.1126.

***N*-(8-Fluoro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-*N*-(2-methoxy-ethyl)-glycine 9{*2*,*3*}.** Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 13% yield; ^{1}H

NMR ($CDCl_3$, 400 MHz) δ 8.02 (s, $1H_{maj}$, C=C*H*), 7.98 (s, $1H_{min}$, C=C*H*), 7.37 (m, $2H_{maj+min}$, Ar-*H*), 7.25 (m, $1H_{maj+min}$, Ar-*H*), 4.36 (s, $2H_{maj}$, (O)CH_2N), 4.18 (s, $2H_{min}$, (O)CH_2N), 3.80 (t, $1H_{min}$, *J*=4.6 Hz, NCH_2CH_2), 3.69 (t, $1H_{min}$, *J*=4.5 Hz, NCH_2CH_2), 3.56 (bs, $2H_{maj}$, NCH_2CH_2 and NCH_2CH_2), 3.36 (s, $3H_{min}$, OCH_3), 3.30 (s, $3H_{maj}$, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.0, 166.4, 166.2, 157.3, 157.2, 150.8, 148.3, 143.7, 143.6, 142.5, 142.4, 125.6, 125.4, 125.3, 124.4, 124.3, 120.5, 120.4, 119.7, 119.5, 71.4, 71.1, 59.2, 59.1, 50.6, 48.8, 47.8; HRMS FAB$(M+H)^+$ Calcd for $C_{15}H_{15}NO_6F$ 324.0883, found 324.0888.

***N*-(2-Methoxy-ethyl)-*N*-(7-methoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9**{*2, 4*}. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 23% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (s, $1H_{maj}$, C=C*H*), 7.95 (s, $1H_{min}$, C=C*H*), 7.47 (t, $1H_{maj+min}$, *J*=8.1 Hz, Ar-*H*), 6.89 (m, $1H_{maj+min}$, Ar-*H*), 6.81 (m, $1H_{maj+min}$, Ar-*H*), 4.35 (s, $2H_{maj}$, (O)CH_2N), 4.20 (bs, $2H_{min}$, (O)CH_2N), 3.91 (s, $3H_{min}$, ArOCH_3), 3.89 (s, $3H_{min}$, ArOCH_3), 3.78 (m, $1H_{min}$, NCH_2CH_2), 3.68 (m, $1H_{min}$, NCH_2CH_2), 3.55 (bs, $2H_{maj}$, NCH_2CH_2 and NCH_2CH_2), 3.36 (s, $3H_{min}$, OCH_3), 3.29 (s, $3H_{maj}$, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.0, 167.3, 167.1, 164.3, 164.2, 159.0, 156.5, 144.9, 144.8, 130.4, 130.3, 120.8, 120.6, 113.8, 112.4, 112.3, 101.0, 100.9, 71.5, 71.4, 59.1, 59.0, 56.3, 52.4, 50.6, 49.0, 47.8; HRMS FAB$(M+H)^+$ Calcd for $C_{16}H_{18}NO_7$ 336.1083, found 336.1082.

***N*-(6-Bromo-8-methoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl)-*N*-(2-methoxy-ethyl)-glycine 9**{*2, 5*}. Flash column chromatography with heptane:EtOAc, containing 1% HOAc, 1:1→1:3, 21% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87 (s, $1H_{maj}$, C=C*H*), 7.85 (s, $1H_{min}$, C=C*H*), 7.27 (m, $1H_{maj+min}$, Ar-*H*), 7.19 (dd, $1H_{maj+min}$, *J*=11.1, 2.0, Ar-*H*), 4.35 (s, $2H_{maj}$, (O)CCH_2N), 4.15 (s, $2H_{min}$, (O)CCH_2N), 3.96 (s, $3H_{maj}$, ArOCH_3), 3.94 (s, $3H_{min}$, ArOCH_3), 3.75 (t, $1H_{min}$, *J*=4.8 Hz, NCH_2CH_2), 3.63, (t, $1H_{min}$, *J*=4.8 Hz, NCH_2CH_2), 3.53 (m, $2H_{maj}$, NCH_2CH_2 and NCH_2CH_2), 3.31 (s, $3H_{min}$, OCH_3), 3.28 (s, $3H_{maj}$, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172. 5, 166.1, 166.0, 157.2, 157.2, 147.9, 147.8, 143.1, 143.0, 142.9, 125.8, 125.7, 122.3, 122.1, 120.0, 119.9, 117.9, 117.5, 117.4, 117.3, 71.3, 71.2, 58.9, 58.8, 56.7, 51.8, 50.3, 48.4, 47.7; HRMS FAB$(M+H)^+$ Calcd for $C_{16}H_{17}NO_7Br$ 414.0188, found 414.0178.

***N*-Benzyl-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9**{*3, 1*}. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 44% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (s, $1H_{min}$, C=C*H*), 7.97 (s, $1H_{maj}$, C=C*H*), 7.57 (m, $4H_{maj+min}$, Ar-*H*), 7.33 (m, $5H_{maj+min}$, Ar-*H*), 4.84 (bs $2H_{min}$, ArCH_2N), 4.60 (bs $2H_{maj}$, ArCH_2N), 4.14 (bs, $2H_{maj}$, (O)CH_2N), 3.96 (bs, $2H_{min}$, (O)CH_2N); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.6, 166.8, 166.5, 158.2, 154.3, 154.2, 144.3, 143.4, 135.4, 134.6, 133.3, 129.1, 129.0, 128.0, 128.5, 128.4,

128.2, 128.8, 125.2, 124.3, 124.2, 118.4, 118.2, 117.0, 116.9, 54.0, 49.4, 46.1; HRMS FAB$(M+H)^+$ Calcd for $C_{19}H_{16}NO_5$ 338.1028, found 338.1031.

***N*-Benzyl-*N*-(3-oxo-3*H*-naphtho[2,1-*b*]pyran-2-carbonyl)-glycine 9**{*3, 2*}. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5→35:60:5; 55% yield; ($CDCl_3$, 400 MHz) δ 8.86 (s, $1H_{min}$, C=C*H*), 8.77 (s, $1H_{maj}$, C=C*H*), 8.19 (m, 1H, Ar-*H*), 8.02 (m, 1H, Ar-*H*), 7.89 (m, 1H, Ar-*H*), 7.62 (m, 2H, Ar-*H*), 7.45-7.23 (m, 6H, Ar-*H*), 4.90 (s, $2H_{min}$, ArCH_2N), 4.66 (s, $2H_{maj}$, ArCH_2N), 4.19 (s, $2H_{maj}$, (O)CH_2N), 4.03 (s, $2H_{min}$, (O)CH_2N); ^{13}C NMR ($CDCl_3$, 100 MHz) 172.8, 167.3, 166.9, 158.6, 154.3, 154.2, 140.4, 139.5, 135.8, 135.3, 134.6, 134.5, 130.6, 130.3, 130.2, 129.5, 129.1, 129.0, 128.9, 128.4, 128.2, 127.9, 127.8, 127.0, 126.6, 126.5, 122.9, 122.5, 121.9, 121.8, 116.5, 112.9, 112.6, 54.0, 49.5, 47.3; HRMS FAB$(M+H)^+$ Calcd for $C_{23}H_{18}NO_5$ 388.1184, found 388.1183.

***N*-Benzyl-*N*-(8-fluoro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9**{*3, 3*}. Flash column chromatography with toluene: EtOAc:HOAc 80:15:5→60:35:5; 23% yield; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.02 (s, $1H_{min}$, C=C*H*), 7.95 (s, $1H_{maj}$, C=C*H*), 7.37-728 (m, ($H_{maj+min}$, Ar-*H*), 4.83 (s, $2H_{min}$, ArCH_2N), 4.60 (s, $2H_{maj}$, ArCH_2N), 4.12 (s, $2H_{maj}$, (O)CCH_2N), 3.94 (s, $2H_{min}$, (O)CCH_2N); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.7, 166.6, 166.3, 157.4, 157.3, 150.8, 148.3, 143.6, 142.9, 142.5, 142.4, 135.7, 135.0, 129.4, 129.2, 129.1, 128.7, 128.6, 128.3, 128.2, 125.5, 125.4, 125.3, 124.5, 124.3, 120.4, 120.2, 119.8, 119.6, 54.2, 49.7, 46.9; HRMS FAB$(M+H)^+$ Calcd for $C_{19}H_{15}NO_5F$ 356.0934, found 356.0931.

***N*-Benzyl-*N*-(7-methoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9**{*3, 4*}. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 34% yield; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.99 (s, $1H_{min}$, C=C*H*), 7.93 (s, $1H_{maj}$, C=C*H*), 7.45 (m, 1H, Ar-*H*), 7.37-7.32 (m, 4H, Ar-*H*), 7.18 (m, 1H, Ar-*H*), 6.88 (m, 1H, Ar-*H*), 6.81 (m, 1H, Ar-*H*), 6.05 (bs, 1H, CO_2H), 4.83 (bs $2H_{min}$, ArCH_2N), 4.60 (bs $2H_{maj}$, ArCH_2N), 4.12 (bs, $2H_{maj}$, (O)CH_2N), 3.95 (bs, $2H_{min}$, (O)CH_2N), 3.88 (s, $3H_{maj+min}$, ArOCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4, 167.3, 167.0, 164.1, 158.8, 158.7, 156.4, 156.3, 144.8, 143.9, 135.6, 135.0, 130.2, 130.0, 129.2, 129.1, 129.0, 128.4, 128.3, 128.2, 127.9, 120.3, 120.2, 113.6, 112.1, 111.9, 100.8, 100.7, 56.1, 54.0, 49.5, 49.3, 46.5; HRMS FAB$(M+H)^+$ Calcd for $C_{20}H_{18}NO_6$ 368.1134, found 368.1140.

***N*-Benzyl-*N*-(6-bromo-8-methoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9**{*3, 5*}. Flash column chromatography with heptane:EtOAc, containing 1% HOAc 2:1→1:3, 23% yield; ^1H NMR (MeOH-d_4, 400 MHz) δ 8.00 (s, $1H_{maj}$, C=C*H*), 7.98 (s, $1H_{min}$, C=C*H*), 7.42-7.17 (m, $7H_{maj+min}$, Ar-*H*), 4.78 (bs, $2H_{min}$, ArCH_2N), 4.65 (bs, $2H_{maj}$, ArCH_2N), 4.08 (bs,

$2H_{min}$, (O)CCH_2N), 3.99 (bs, $2H_{maj}$, (O)CCH_2N), 3.95 (s, $3H_{maj}$, ArOCH_3), 3.94 (s, $3H_{min}$, ArOCH_3); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 172.4, 171.5, 168.0, 167.9, 163.7, 158.8, 149.2, 144.2, 144.1, 143.2, 143.1, 138.5, 137.0, 136.8, 131.6, 131.5, 129.8, 129.3, 129.1, 128.8, 128.7, 128.6, 126,7, 123.7, 123.3, 122.3, 121.3, 119.0, 118.2, 115.1, 111.3, 111.2, 103.3, 103.1, 99.0, 66.1, 58.5, 58.2, 57.2, 56.8, 56. 7, 54.8; HRMS FAB$(M+H)^+$ Calcd for $C_{20}H_{17}NO_6Br$ 446.0239, found 446.0236.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9{*4*, *1*}**. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 54% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.49 (s, $1H_{maj}$, C=C*H*), 8.35 (s, $1H_{min}$, C=C*H*), 7.46 (m, 1H, Ar-*H*), 7.20-7.07 (m 4H, Ar-*H*), 7.00 (m, 1H, Ar-*H*), 6.93 (s, 1H, Ar-*H*), 6.83 (bt, 1H, *J*=7.9 Hz, Ar-*H*), 6.68 (bt, 1H, *J*=7.9 Hz, Ar-*H*), 4.29 (bs, $2H_{maj}$, (O)CCH_2), 3.89 (bs, $2H_{min}$, (O)CCH_2, and $2H_{min}$, NCH_2CH$_2$), 3.69 (m, $2H_{maj}$, NCH_2CH$_2$), 3.17 (m, $2H_{min}$, NCH$_2$CH_2), 3.00 (m, $2H_{maj}$, NCH$_2$CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.7, 167.3, 158.4, 153.6, 144.0, 136.1, 132.7, 129.3, 127.0, 124.6, 124.3, 122.7, 122.1, 119.4, 118.8, 117.7, 117.5, 116.3, 111.6, 110.8, 51.3, 48.9, 32.0, 29.2, 24.3, 22.8; HRMS FAB$(M+H)^+$ Calcd for $C_{22}H_{19}N_2O_5$ 391.1239, found 391.1300.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(3-oxo-3*H*-naphtho[2,1-*b*]pyran-2-carbonyl)-glycine 9{*4*, *2*}**. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 36% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.46 (s, $1H_{min}$), 8.37 (s, $1H_{maj}$), 8.03 (s, 1H, Ar-*H*), 7.93 (d, 1H, *J*=8.2 Hz, Ar-*H*), 7.81 (t, 2H, *J*=8.3 Hz, Ar-*H*), 7.60 (t, 2H, *J*=7.8 Hz, Ar-*H*), 7.53 (t, 2H, *J*=7.6 Hz, Ar-*H*), 7.12 (m, 4H, Ar-*H*), 6.50 (m, 1H, Ar-*H*), 6.22 (m, 1H, Ar-*H*), 4.33 (s, $1H_{maj}$, (O)CH_2N), 4.00 (s, $1H_{min}$, (O)CH_2N), 3.92 (s, $1H_{min}$, NCH_2CH$_2$), 3.81 (s, $1H_{maj}$, NCH_2CH$_2$), 3.19 (bs, $1H_{min}$, NCH$_2$CH_2), 2.96 (s, $1H_{maj}$, NCH$_2$CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz,) δ 171.8, 167.9, 158.3, 154.0, 141.2. 135.6, 134.3, 130.1, 129.3, 128.8, 128.6, 126.8, 126.4, 124.3, 121.8, 121.5, 121.4, 119.1, 117.0, 116.3, 112.6, 111.3, 111.1, 51.9, 50.4, 32.0, 29.2, 24.6, 22.9, 14.3; HRMS FAB$(M+H)^+$ Calcd for $C_{26}H_{21}N_2O_5$ 441.1450, found 441.1452.

***N*-(8-Fluoro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-*N*-[2-(1*H*-Indol-3-yl)-ethyl]-glycine 9{*4*, *3*}**. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 36% yield; ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.45-7.25 (m, 1H, Ar-*H*), 7.17 (m, 2H, Ar-*H*), 7.10 (m, 2H, Ar-*H*), 7.00 (m, 2H, Ar-*H*), 6.75 (m, 1H, Ar-*H*), 6.63 (m, 1H, Ar-*H*), 4.28 (bs, $2H_{maj}$, (O)CCH_2), 3.95 (bs, $2H_{min}$, (O)CCH_2), 3.81 (t, $2H_{min}$, *J*=7.1 Hz, NCH_2CH$_2$), 3.71 (t, $2H_{maj}$, *J*=6.0 Hz, NCH_2CH$_2$), 3.12 (t, $2H_{min}$, *J*=7.3 Hz, NCH$_2$CH_2), 2.98 (t, $2H_{maj}$, *J*=6.0 Hz, NCH$_2$CH_2); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 172.1, 167.8, 158.1, 151.4, 148.9, 143.9, 142.9, 142.8, 138.2, 137.9, 130.0, 129.3, 128.4, 126.4, 126.3, 126.2, 125.9, 125.8, 125.7,

125.9, 125.1, 124.1, 122.5, 121.1, 119.8, 119.7, 119.6, 119.4, 118.4, 112.5, 112.4, 112.2, 52.2, 49.8, 25.1, 24.1; HRMS FAB$(M+H)^+$ Calcd for $C_{22}H_{18}N_2O_5F$ 409.1199, found 409.1297.

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(7-methoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine 9**{*4, 4*}. Flash column chromatography with toluene:EtOAc:HOAc 80:15:5→60:35:5; 34% yield; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 8.49 (s, $1H_{maj}$), 8.38 (s, $1H_{min}$), 7.19 (m, 3H, Ar-*H*), 7.07 (m, 1H, Ar-*H*), 6.85 (m, 3H, Ar-*H*), 6.70 (m, 2H, Ar-*H*), 6.60 (d, 1H, *J*=2.2 Hz, Ar-*H*), 4.27 (s, $1H_{maj}$, (O)CH_2N), 3.92 (s, $1H_{minj}$, (O)CH_2N), 3.82 (m, 3H, ArOCH_3, and $2H_{min}$, NCH_2CH$_2$), 3.70 (bs, $2H_{maj}$, NCH_2CH$_2$), 3.15 (bs, $2H_{min}$, NCH$_2$CH_2), 2.98 (bs, $2H_{maj}$, NCH$_2$CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.8, 167.8, 163.7, 158.8, 155.7, 144.3, 138.0, 136.2, 130.3, 129.2, 128.4, 127.0, 125.4, 124.3, 121.9, 119.3, 118.9, 117.6, 113.0, 111.6, 111.5, 110.9, 100.2, 55.9, 51.4, 49.1, 24.3, 21.6; HRMS FAB$(M+H)^+$ Calcd for $C_{23}H_{21}N_2O_6$ 421.1399, found 421.1389.

***N*-(6-Bromo-8-methoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl)-*N*-[2-(1*H*-Indol-3-yl)-ethyl]-glycine 9**{*4, 5*}. Flash column chromatography with heptane:EtOAc, containing 1% HOAc, 3:1→2:1→1:2, 50% yield; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 8.68 (s, $1H_{maj}$), 8.60 (s, $1H_{min}$), 7.20-7.03 (m, 5H, Ar-*H*), 6.94 (d, 1H, *J*=1.8 Hz, Ar-*H*), 6.82 (t, 1H, *J*=7.6 Hz, Ar-*H*), 6.64 (t, 1H. *J*=7.5 Hz, Ar-*H*), 6.48 (s, 1H, Ar-*H*), 6.44 (d, 1H, *J*=1.7 Hz, Ar-*H*), 4.25 (s, $1H_{maj}$, (O)CH_2N), 3.90 (s, $1H_{min}$, (O)CH_2N), 3.82 (m, 3H, ArOCH_3, and $2H_{min}$, NCH_2CH$_2$), 3.65 (bs, $2H_{maj}$, NCH_2CH$_2$), 3.12 (m, $2H_{min}$, NCH$_2$CH_2), 2.90 (m, bs $2H_{maj}$, NCH$_2$CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.5, 166.9, 157.3, 147.2, 142.8, 142.2, 136.1, 129.2, 128.4, 127.0, 124.7, 123.8, 122.7, 121.9, 119.3, 119.1, 117.4, 117.2, 116.7, 111.6, 110.7, 56.6, 51.2, 48.9, 24.1, 22.8; HRMS FAB$(M+H)^+$ Calcd for $C_{23}H_{20}N_2O_6Br$ 499.0504, found 499.0502.

Example 5: Solid-Phase Synthesis of N-Alkylated and N-Acylated amino acids

Resin-bound phenylalanine (23). DIC (201 μL, 1.30 mmol) was added to a solution of Fmoc-Phe-OH (604 mg, 1.56 mmol) and HOBt (140 mg, 1.04 mmol) in dry THF (4 mL). After 1 h, the activated amino acid and DMAP (21 mg, 0.17 mmol, dissolved in dry THF, 1 mL) were added to **2** (2.0 g, 0.52 mmol, pre-swollen in dry THF) and the mixture was agitated at ambient temperature over night. After filtration the resin was washed with THF, MeOH and THF (50 mL of each solvent), and dried over vacuum. A solution of 20% piperidine in DMF was added to the resin (pre-swollen in DMF) and the mixture was agitated for 30 min. After filtration the resin was washed with portions of DMF, MeOH, THF and dry THF, and then dried under vacuum.

Solid-phase reductive alkylation of 40. Resin **40** was split into portions and subjected to different conditions for reductive alkylation. These, and the results from the reductive alkylation, are summarized under entries 1-6 in Table B, and described in detail below.

Table C. Reductive alkylation of **40** with *p*-fluorobenzaldehyde using $NaBH_3CN$ as reducing agent.

Entry	Reaction conditions			Yield (%)[a]		
	Solvent	Ratio **23**:*p*-F-PhCHO: $NaBH_3CN$	Reaction time	**24a/25a**	**25a**	**26a**
1	TMOF[b]:HOAc (99:1)	1:10:10	30min+10min	64		
2	THF:HOAc:H_2O (90:5:5)	1:1.2:0.9	5min+3h	55		
3	MeOH:HOAc (99:1)	1:1.1:5	1h+3h	78		
4	MeOH:HOAc (99:1)	1:1.5:5	1h+3h		8	68
5	MeOH:HOAc (99:1)	1:3:7	1h+3h		10	71
6[c]	MeOH:HOAc (99:1)	1:3:7	1h+3h		<2	92

[a] Conversion of **40** as revealed by gel-phase ^{19}F NMR spectroscopy.
[b] TMOF = Trimethylorthoformate.
[c] The aldehyde was allowed to react with **40** for 1 h, excess aldehyde was then removed by filtration prior to addition of $NaBH_3CN$.

Entry 1. p-Fluorobenzaldehyde (42 µL, 390 µmol) was added to **40** [150 mg, 39 µmol, pre-swollen in dry trimethyl orthoformiate (TMOF)] in TMOF (2 mL), and the mixture was agitated for 40 min. $NaBH_3CN$ (25 mg, 390 µmol) dissolved in TMOF (1 mL) and HOAc (18 µL) was added and the mixture was agitated for 10 min. After filtration the resin was washed with portions of TMOF, MeOH and THF (2x10 mL of each solvent) and dried under vacuum.

Entry 2. p-Fluorobenzaldehyde (5 µL, 47 µmol) was added to **40** [150 mg, 39 µmol, pre-swollen in THF:HOAc:H_2O (90:5:5)] in THF:HOAc:H_2O (90:5:5, 1 mL), and the mixture was agitated for 10 min. A solution of $NaBH_3CN$ (35 µL, of a 1 M solution in THF, 35 µmol) was added and the mixture was agitated for 3 h. After filtration, the resin was washed with portions of THF, H_2O, MeOH and THF (10 mL of each solvent), and dried under vacuum.

Entry 3. p-Fluorobenzaldehyde (4.6 µL, 43 µmol) was added to a suspension of **40** (150 mg, 39 µmol, pre-swollen in MeOH containing 1% HOAc) in MeOH containing 1% HOAc (1 mL), and the mixture was agitated for 60 min. $NaBH_3CN$ (12 mg, 195 µmol) dissolved in MeOH (200 µL) was added and the mixture was agitated for 3 h. After filtration, the resin was washed with MeOH, H_2O, MeOH and THF (10 mL of each solvent), and dried under

vacuum.

Entries 4 and 5. Reductive alkylation was performed as described for entry 3 using **40** (100 mg, 26 μmol), but with larger amounts of *p*-fluorobenzaldehyde (4.2 μL, 39 μmol for entry 4; 8.4 μL, 78 μmol for entry 5) and $NaBH_3CN$ (8 mg, 130 μmol for entry 4; 11 mg, 182 μmol for entry 5).

Entry 6. p-Fluorobenzaldehyde (8.4 μL, 78 μmol) was added to a suspension of **40** (100 mg, 26 μmol, pre-swollen in MeOH containing 1% HOAc) in MeOH containing 1% HOAc (1 mL), and the mixture was agitated for 60 min. The solution was removed by filtration and additional MeOH containing 1% HOAc (1 mL) was added followed by $NaBH_3CN$ (11 mg, 182 μmol) dissolved in MeOH (200 μL). The mixture was agitated for 3 h after which the resin was filtered and washed with MeOH, H_2O, MeOH and THF (10 mL of each solvent), and dried under vacuum.

Gel-phase ^{19}F NMR spectroscopy of the resins was recorded as described in general methods and materials.

Acylation of resins obtained by reductive alkylation of 40. Resins obtained by reductive alkylation as described under entries 4 - 6 above (each 100 mg, 26 μmol) were suspended in dry CH_2Cl_2 (1 mL). DIPEA (13 μL, 78 μmol) was added, followed by 4-fluoronaphtoyl chloride (16 mg, 78 μmol) dissolved in dry CH_2Cl_2 (200 μL), after which the mixture was agitated at ambient temperature for 2 h. The solution was filtered off, and the resin was washed with CH_2Cl_2, MeOH and THF (20 mL portions of each solvent). The resin was then dried under vacuum. Gel-phase ^{19}F NMR spectroscopy of the resins was recorded as described in general methods and materials.

***N*-(4-Fluorobenzyl)-*N*-(4-fluoronaphtoyl)-phenylalanine (44).** Solid-phase reductive alkylation of **40** was performed as described in entry 6 above by treatment of resin **40** (1.0 g, 0.26 mmol) with *p*-fluorobenzaldehyde (84 μL, 0.78 mmol) and $NaBH_3CN$ (114 mg, 1.82 mmol). Acylation of the resulting resin was accomplished using 4-fluoronaphtoyl chloride (162 mg, 0.78 mmol) and DIPEA (130 μL, 0.78 mmol) as described above. Compound **44** was cleaved from the resin using aqueous LiOH (1 M, 4 mL) in THF:MeOH:H_2O (3:1:1; 40 mL) at ambient temperature for 2 h. After filtration and subsequent washing of the resin with HOAc and THF (80 mL of each solvent), the filtrate was concentrated and finally co-concentrated from toluene (3x50 mL). The residue was dissolved in a mixture of EtOAc (30 mL) and aqueous HCl (0.05 M, 10 mL). The water phase was separated and acidified with aqueous HCl (1 M) and extracted with EtOAc

(2x30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The crude product was purified by flash column chromatography (heptane:EtOAc 4:1Ò2:1, containing 1% HOAc) to give **44** (88 mg, 76% yield, based on the initial loading capacity of the resin). ^{1}H NMR (DMSO-d_6, 300 MHz, 420 K) 8.07 (d, 1H, J=8.2 Hz, Ar-H), 7.84 (d, 1H, J=8.0 Hz, Ar-H), 7.66-7.50 (dt, 2H, J=7.6, 1.2 Hz, Ar-H), 7.26-7.05 (m, 9 H, Ar-H), 6.90 (t, 2 H, J=8.7 Hz, Ar-H), 4.63 (dd, 1H, J=8.3, 6.2 Hz, Phe-H_α), 4.55 (m, 1H, Phe-H_β), 4.27 (bd, 1H, J=15.6 Hz, Phe-H_β), 3.43 (dd, 1H, J=14.0, 6.1 Hz, $ArCH_2N$), 3.26 (m, 1H, $ArCH_2N$); ^{13}C NMR ($CDCl_3$, 100 MHz) 174.8, 171.7, 163.8, 161.3, 160.7, 158.2, 138.0, 130.5, 129.6, 129.5, 129.2, 129.0, 128.7, 128.6, 128.4, 127.3, 127.2, 125.2, 121.1, 115.7, 115.5, 108.8, 60.6, 54.8, 54.8, 34.9; HRMS (EI$^+$) Calcd for $C_{27}H_{22}NO_3F_2$ 446.1567, found 446.1573.

Example 6 : Direct Binding Assay

The affinity of the low molecular weight compounds as synthesized in the above Examples, for periplasmic chaperones PapD and FimC were investigated using a direct binding assay on BIACORE 3000.

Methods

PapD (50 μg/mL in 10 mM NaAc pH 5.5) and FimC (50 μg/mL in 10 mM NaAc pH 5.5) were immobilized on Sensor Chip CM5 using a standard thiol coupling procedure. This procedure was also employed for coupling of non-target proteins. Immobilization levels of 6-8 000 RU were obtained. Unmodified dextrane in one of the flow cells was used as reference surface.

The compounds were prepared as described in Examples 1 and 2 and diluted from 10 mM DMSO stock solutions to a final concentration of 100 μM or 10 μM in running buffer (67 mM phosphate buffer (9.6 g $Na_2HPO_4 \cdot 2H_2O$, 1.7 g KH_2PO_4, 4.1 g NaCl, 1000 mL H_2O), 3.4 mM EDTA, 0.01% Tween, 5% DMSO, pH 7.4.) so that the concentrations of DMSO and buffer substances were carefully matched. The compounds were injected (flow rate was 30 μl/min at 25°C) and the binding of the compounds to the immobilized chaperone proteins was observed on real time. The surface was regenerated by injection of 10 mM glycine•HCl, pH 2.0. To avoid carry over, the flow system was washed with a 1:1 mixture of DMSO and H_2O.

For screening of the affinity of the compounds for PapD and FimC, the compounds were injected (flow rate was 30 μl/min at 25°C) at a concentration of 100 μM in duplicate or triplicate and in random order. The surface was regenerated by injection of 10 mM glycine•HCl, pH 2.0. To avoid carry over, the flow system was washed with a 1:1 mixture

of DMSO and H_2O. Reference chemicals were used as negative controls.

For the estimation of K_D, the compounds were injected in concentration series of 1.0 μM-100 μM, and association and dissociation kinetics were studied. Selectivity data was obtained by injecting the compounds over both target and non-target, *i.e.* Protein A, Streptavidin, and Anti-myoglobin mAb, surfaces.

Results

Table D: Affinity of Compounds for PapD and FimC.

Compound	Response Units	
	PapD	FimC
N-[2-(1H-Indol-3-yl)-ethyl]-N-(naphtalene-2-carbonyl)-tyrosine **19** {3,3}	110	120
N-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-tyrosine **17**{*3*, *1*}.	40	48
N-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(3-oxo-3*H*-naphtho[2,1-*b*]pyran-2-carbonyl)-glycine **9**{*4*, *2*}.	30	38
N-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-lysine **17**{*4*, *1*}.	20	17
N-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(3-methyl-butyryl-carbonyl)-tyrosine **19** {3,4}	--	18
N-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(naphtalene-2-carbonyl)-glycine **19**{*1*, *2*}.	17	22

For compounds showing strong and medium affinity for PapD and FimC (N-[2-(1H-Indol-3-yl)-ethyl]-N-(naphalene-2-carbonyl)-tyrosine **19** {3,3},*N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-tyrosine **17**{*3*, *1*}, K_D was estimated to 1-100 μM.

No significant non-specific binding of the compounds to the non-target proteins were observed.

Example 7: Inhibition Assay Using FimCH Reconstitution Assay

In order to conclusively identify the compounds that inhibit a pilus chaperone, Applicants conducted a reconstitution assay using FimCH and PapDG.

Methods.

The FimCH complex was brought to 3M Urea in 20mM MES pH 6.8 and injected onto a 1ml Source 15S Pharmacia column (1ml/min flow rate) and pure FimH was collected in the Flow Through. The PapDG complex was brought to 5M Urea in 20mM MES pH 6.8 and injected onto a 1ml Source 15S Pharmacia column (1ml/min flow rate) and pure Pap D was collected in the Flow Through.

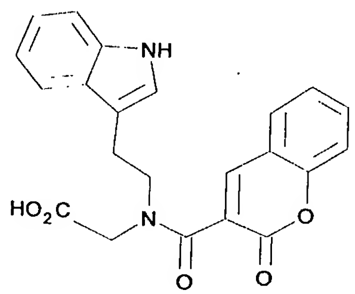
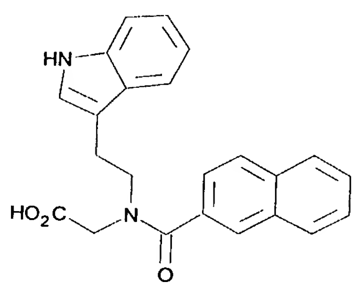
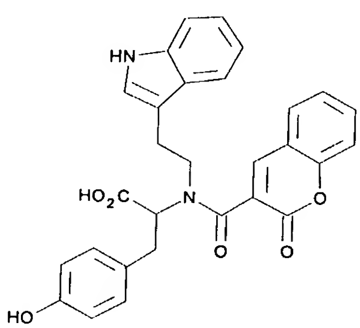
For wild type control assay, PapG in 5M Urea was diluted 10fold volume into 20mM MES pH 5.8 containing an equimolar amount of PapD. This was reinjected onto the 1ml Source 15S column and the reconstituted PapDG complex was eluted using 40mM NaCl and the excess PapD was eluted using 65mM NaCl. Using the Unicorn program for the Pharmacia AKTA, the area under the PapDG peak was integrated and this was calculated to be 100% reconstitution. The same process was repeated for the wild type control assay for FimCH except that FimH was in 3M Urea was diluted 10fold volume into 20mM MES pH 5.8 containing an equimolar amount of PapD.

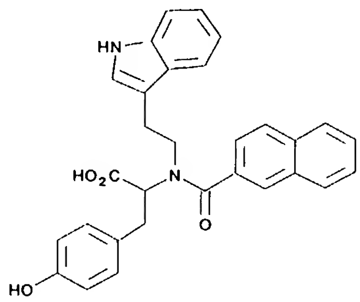
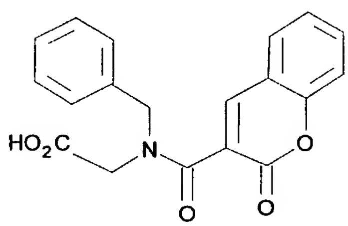
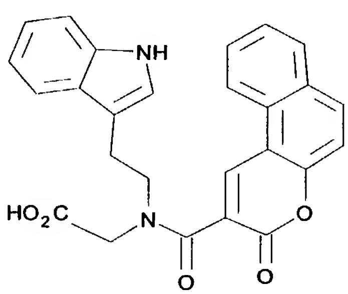
The candidate compounds were synthesized as described in Examples 1 and 2. For the tested compounds, a 38 M excess was preincubated with the PapD (or FimC) for 15 minutes and the same reconstitution and PapDG (or FimCH) separation was performed. Binding of the compounds to the chaperones in the column were detected using UV light (UVA 280). Peak areas were calculated as a percent of wild type.

Results

Table E represents the library of compounds evaluated for the inhibition of formation of the complex between PapD and PapG; the % inhibition of the complex between PapD and PapG at an inhibitor/PapD ratio of 38. Compound 9{4, 1} was also evaluated as an inhibitor of FimCH complex formation. It gives 18% inhibition at a 69 fold excess as compared to FimC and 60% inhibition at a 207 fold excess.

TABLE E

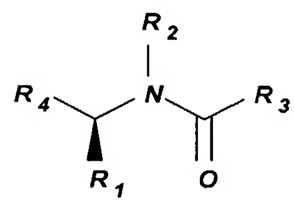
Compound	Compound Reference	% Inhibition at Inhibitor/PapD ratio 38
	9{4, 1}	24.4
	19{1, 2}	16.8
	17{3, 1}	14.5

	19{3, 3}	47.3
	9{3, 1}	31.5
	9{4, 2}	27.3

Other features, objects and advantages of the present invention will be apparent to those skilled in the art. The explanations and illustrations presented herein are intended to acquaint others skilled in the art with the invention, its principles, and its practical application. Those skilled in the art may adapt and apply the invention in its numerous forms, as may be best suited to the requirements of a particular use. Accordingly, the specific embodiments of the present invention as set forth are not intended as being exhaustive or limiting of the present invention.

WE CLAIM:

1. A compound of the formula:



wherein each of R_1, R_2 and R_3 is independently a substituted or unsubstituted alkyl (C_{1-10}), substituted or unsubstituted acyl (C_{2-15}), substituted or unsubstituted aryl (C_{6-14}), substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl (C_{7-15}), substituted or unsubstituted heteroarylalkyl or substituted or unsubstituted heterocycloalkyl;

R_4 is a carboxy ($-CO_2H$), carboxamide ($-CONH_2$), aldehyde (-CHO), boronate ($-B(OH)_2$), phosphonate ($PO(OH)_2$) or ketone (-COR) wherein R is a halogenated or unsubstituted alkyl (C_{1-3}); and the salts, esters and amines thereof.

2. The compound of claim 1 wherein substituents on any alkyl or alkylene moiety is selected from the group consisting of halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy, carboxamido, cyano, carbonyl, NO_2, alkylthio, alkylthiol, sulfoxide, sulfone, acylamino, amidino, phenyl, benzyl, heteroaryl, phenoxy, benzyloxy, heteroaryloxy, substituted phenyl, benzyl, heteroaryl, phenoxy, benzyloxy, heteroaryloxy and NR' R" wherein each R' and R"is independently H, alkyl or arylalkyl.

3. The compound of claim 2 wherein R_1 is selected from side chains of amino acid residues; R_2 is substituted or unsubstituted alkyl, arylalkyl, heteroarylalkyl, or heterocycloalkyl; R_3 is substituted or unsubstituted alkyl, aryl, or heteroaryl; and R_4 is a carboxyl group ($-CO_2H$), carboxamide ($-CONH_2$), aldehyde (-CHO), boronate ($-B(OH)_2$) or phosphonate ($PO(OH)_2$).

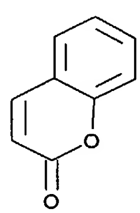
4. The compound of claim 3 wherein R^1 is selected from the side chains of hydrophobic aromatic, hydrophobic aliphatic, polar and charged amino acid residues; R_2 is substituted or unsubstituted arylalkyl or substituted or unsubstituted

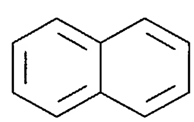
heteroarylalkyl; R_3 is substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl; and R_4 is $-CO_2H$, $-CONH_2$ or -CHO.

5. The compound of claim 4 wherein R_1 is selected from the side chains of hydrophobic aromatic, hydrophobic aliphatic and polar amino acid residues; R_2 is substituted or unsubstituted arylalkyl or substituted or unsubstituted heteroarylalkyl; R_3 is substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl; and R_4 is $-CO_2H$ or -CHO.

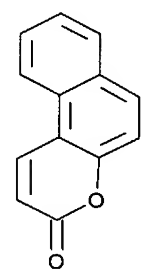
6. The compound of claim 5 wherein R_1 is selected from hydrophobic aromatic and hydrophobic aliphatic amino acid residues; R_2 is substituted or unsubstituted arylalkyl or substituted or unsubstituted heteroarylalkyl; R_3 is substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl; and R_4 is $-CO_2H$.

7. The compound of claim 6 wherein R_1 is hydrogen, benzyl, 4-aminobutyl or p-hydroxy-benzyl; R_2 is $(CH_2)_mA$ wherein m is between 0 and 3 and A is n-butyl, phenyl and 3-indolyl; R_3 is isobutyl,



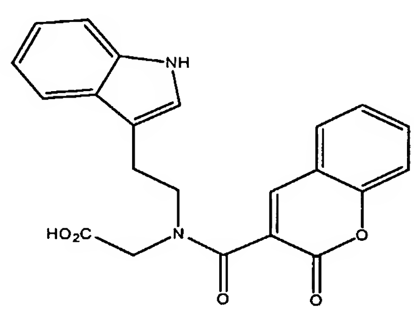


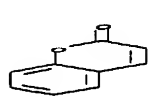
AND

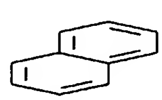


and R_4 is carboxyl.

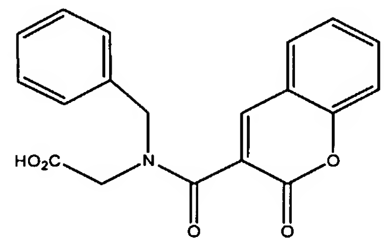
8. The compound of claim 7 wherein said compound is



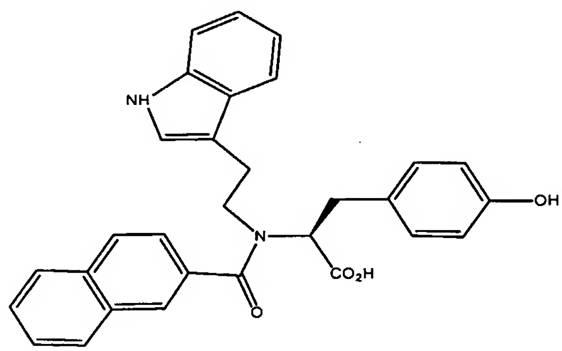




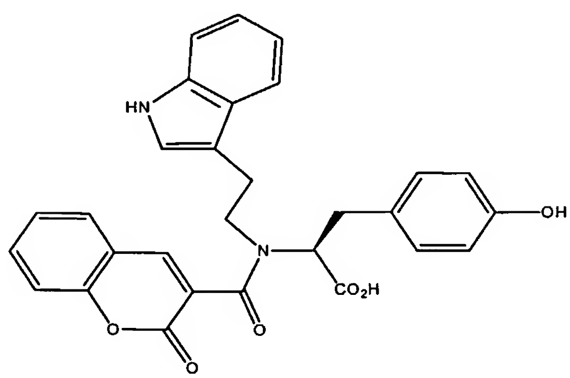
9. The compound of claim 7 wherein said compound is



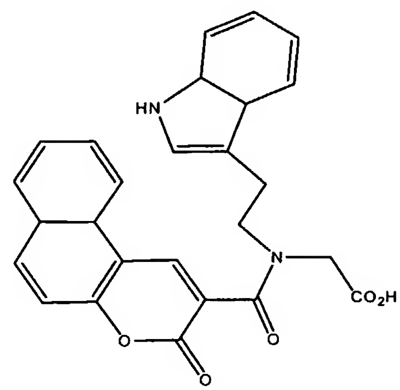
10. The compound of claim 7 wherein said compound is



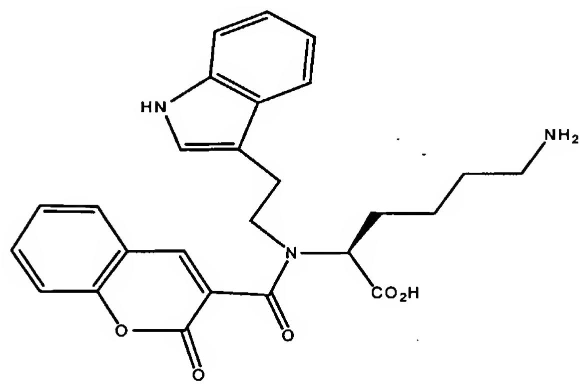
11. The compound of claim 7 wherein said compound is



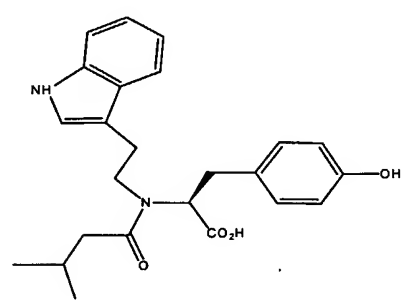
12. The compound of claim 7 wherein said compound is



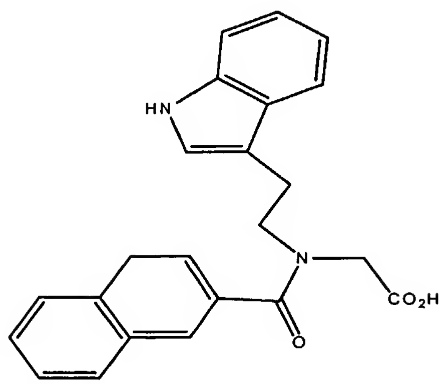
13. The compound of claim 7 wherein said compound is



14. The compound of claim 7 wherein said compound is



15. The compound of claim 7 wherein said compound is



16. The compound of claim 1 wherein each R_1, R_2 and R_3 is independently an amino acid residue side chain and R_4 is carboxyl or aldehyde.

17. The compound of claim 16 wherein the amino acid residue side chain is selected from the group consisting of hydrogen, p-hydroxybenzyl, 2-(3-indolyl)-ethyl, benzyl, 5-imidazole, isopropyl, isobutyl, 2-methylpropyl, methyl and 2-thiomethylethyl.

18. The compound of claim 17 wherein R_1 is hydrogen or p-hydroxybenzyl.

19. The compound of claim 18 wherein R_1 is p-hydroxybenzyl.

20. The compound of claim 18 wherein R_1 is hydrogen.

21. The compound of any one of claims 1-20 contained in a mixture of its stereoisomers.

22. The compound of any one of claims 1-20 in optically pure form.

23. The compound of any one of claims 1-20 wherein said compound exhibits antibacterial activity against a Gram-negative bacterium selected from the group consisting of *Escherichia coli, Haemophilus influenzae, Salmonella enteriditis, Salmonella typhimurium, Bordetella pertussis, Yersinia pestis, Yersinia enterocolitica, Helicobacter pylori* and *Klebsiella pneumoniae.*

24. The compound of claim 23 wherein said compound exhibits antibacterial activity by binding to a pilus chaperone thereby exhibiting antibacterial activity against a Gram-negative bacterium.

25. The compound of claim 24 wherein the pilus chaperone is a PapD chaperone.

26. The compound of claim 24 wherein the pilus chaperone is a FimC chaperone.

27. A method of treating a Gram-negative infection in a subject which method comprises administering to a subject in need thereof an effective amount of the compound of claim 23.

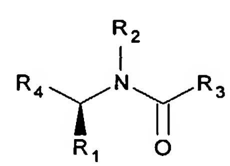
28. A method of preventing or inhibiting the attachment of a Gram-negative organism to host tissues in a mammal, said method comprising administering an effective amount of the compound of claim 23 to said mammal thereby inhibiting pili assembly.

29. A method of preventing or inhibiting biofilm formation, said method comprising administering a compound of claim 23 to an environment or surface containing Gram-negative bacteria.

30. A method of preventing or inhibiting bacterial colonization by a Gram-negative organism in a mammal, said method comprising administering the compound of claim 23 to said mammal.

31. A pharmaceutical composition comprising the compound of claim 23 in combination with a pharmaceutically acceptable carrier.

32. A process for the preparation of a compound of the formula

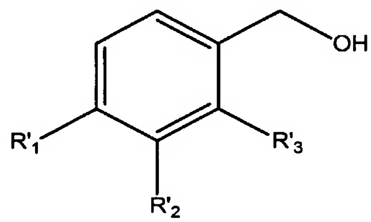


wherein R_1 is hydrogen; R_2 is $(CH_2)_mA$ wherein m is between 0 and 3 and A is n-butyl, 2-methoxyethyl, benzyl and 2-(3-indolyl)-ethyl; R_3 is coumarin; and R_4 is carboxyl,

said method comprising condensing a compound 6 of Reaction Scheme 1 with a salicyaldehyde selected from the group consisting of the compounds listed in Table A.

33. The process of claim 32 wherein the process occurs in solution.

34. A linker compound of the formula:



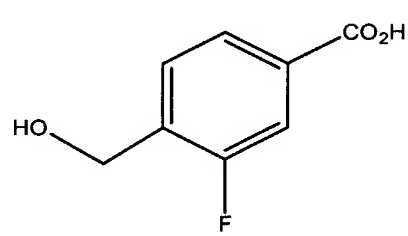
wherein R'_1 is $-CO_2H$, $-(CH_2)_nCO_2H$ or $-O(CH_2)_nCO_2H$ wherein n is between 1 and 10; and R'_2 and R'_3 is independently fluorine or hydrogen provided that when either R'_2 or R'_3 is fluorine, the other is hydrogen.

35. The linker compound of claim 34 wherein R'_2 is hydrogen and R'_3 is fluorine.

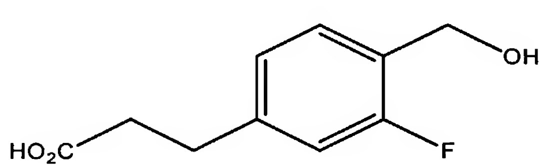
36. The linker compound of claim 35 wherein n is between 1 and 5.

37. The linker compound of claim 36 wherein n is 1 or 2.

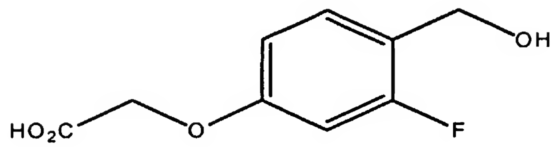
38. The linker compound of claim 37 wherein the linker compound is



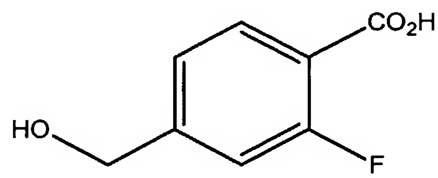
39. The linker compound of claim 37 wherein the linker compound is



40. The linker compound of claim 37 wherein the linker compound is



41. The linker compound of claim 34 wherein the linker compound is



42. A process of synthesizing a linker compound, said process comprising:

a. hydrolyzing one of the ester moieties of dimethyl-2-fluoroterephtalate;

b. reducing the remaining ester; and

c. separating the two regioisomers.

43. The linker compound produced by the process of claim 42.

44. The process of claim 42 wherein the remaining ester is reduced with $LiBH_4$.

45. The process of claim 42 further comprising:

d. protecting a fluorinated 4-(hydroxymethyl) benzoic acid produced by the process of claim 42 thereby forming a benzyl ester;

e. oxidizing the hydroxymethyl group and condensing the resulting aldehyde with an ethyl acetate compound;

f. reducing the product of step (e);

g. reducing the carboxyl group of the product of step (f); and

h. hydrolyzing the ethyl ester.

46. The compound produced by the process of claim 45.

47. A process of synthesizing a linker compound, said process comprising:

a. dealkylating a 2-fluoro-4-propoxybenzoic acid;

b. reducing the carboxylic acid of the product of step (a) thereby producing a hydroxymethylphenol compound;

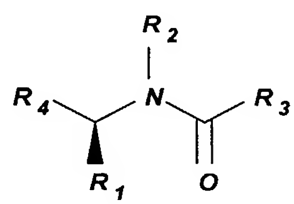
c. alkylating the phenolic hydroxyl group of the hydroxymethylphenol compound; and

d. hydrolyzing the product of step (c) under basic conditions.

48. The linker compound produced by the process of claim 47.

49. The process of claim 32 wherein the process occurs on solid phase whereby a linker compound of any one of claims 38-41, 43, 45 or 48 is interposed between a solid support and said compound 6 of Reaction Scheme 1.

50. A process for the solid-phase synthesis of a compound of the formula:



wherein R_1 is hydrogen, R_2 is $(CH_2)_mA$ wherein m is between 0 and 3 and A is selected from the group consisting of n-butyl, benzyl and 2-(3-indolyl)-ethyl, R_3 is coumarin and R_4 is carboxyl, said process comprising the steps of:

a. affixing a linker compound of any one of claims 38-41, 43, 45 or 48 onto a solid support to give a benzylic alcohol;

b. subjecting the benzylic alcohol to acylation with bromoacetic acid;

c. subjecting the bromoacetate to a nucleophilic substitution with an amine;

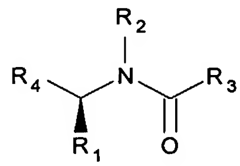
d. acylating with ethyl malonyl chloride thereby forming a N-alkyl-N-(malonamic acid ethyl ester)-glycine derivative;

e. condensing the N-alkyl-N-(malonamic acid ethyl ester)-glycine derivative with a salicyaldehyde; and

f. cleaving the compound from the linker compound under acidic or basic conditions.

51. The process of claim 50 wherein step (b) is repeated once.

52. A process for the solid-phase synthesis of a compound of the formula

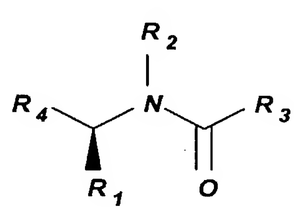


wherein R_1 is selected from side chains of any amino acid residue provided that R_1 is not hydrogen, R_2 and R_3 is independently a substituted or unsubstituted alkyl (C_{1-10}), substituted or unsubstituted acyl (C_{2-15}), substituted or unsubstituted aryl (C_{6-14}), substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl (C_{7-15}), substituted or unsubstituted heteroarylalkyl or substituted or unsubstituted heterocycloalkyl and R_4 is carboxyl, said process comprising the steps of:

a. affixing a linker compound of any one of claims 38-42, 43, 45 or 48 onto a solid support to give a benzylic alcohol;
b. coupling a Fmoc-protected amino acid to the benzylic alcohol thereby producing a amino acid functionalized resin;
c. removing the Fmoc-protecting group from the α-amino acid group of the product of step (b);
d. alkylating the α-amino group of the product of step (c) by reductive alkyation;
e. removing excess aldehyde from the amino acid functionalized resin;
f. acylating with an acid chloride thereby producing a N-(alkylated)-N-(acylated)-amino acid derivative; and
g. cleaving the compound from the linker under acidic or basic conditions.

53. In a method of synthesizing a combinatorial library, an improvement comprising affixing a linker compound of any one of claims 38-41, 43, 45 or 48 onto a solid support.

54. A library of compounds which are candidates targeted for antibacterial properties comprising at least two different compounds having the formula:



wherein each of R_1, R_2 and R_3 is independently a substituted or unsubstituted alkyl (C_{1-10}), substituted or unsubstituted acyl (C_{2-15}), substituted or unsubstituted aryl (C_{6-14}), substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl (C_{7-15}), substituted or unsubstituted heteroarylalkyl or substituted or unsubstituted heterocycloalkyl; and

R_4 is a carboxy ($-CO_2H$), carboxamide ($-CONH_2$), aldehyde (-CHO), boronate ($-B(OH)_2$), phosphonate ($PO(OH)_2$) and ketone (-COR) wherein R is a halogenated or unsubstituted alkyl (C_{1-3}), wherein any of the candidate compounds are retrievable and analyzable for said antibacterial properties.

55. The library of claim 54 wherein each R_1 is independently hydrogen, p-hydroxybenzyl, benzyl or 2-(3-indolyl)-ethyl.

56. The library of claim 55 wherein each R_1 is hydrogen.

57. The library of claim 55 wherein each R_1 is p-hydroxybenzyl.

58. The library of claim 56 or 57 wherein the library comprises at least five different compounds.

59. The library of claim 56 or 57 wherein the library comprises at least ten different compounds.

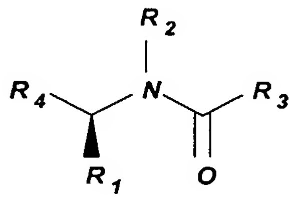
60. The library of any one of claims 55-59 wherein said library of compounds exhibit antibacterial activity against a Gram-negative bacterium selected from the group consisting of *Escherichia coli, Haemophilus influenzae, Salmonella enteriditis, Salmonella typhimurium, Bordetella pertussis, Yersinia pestis, Yersinia enterocolitica, Helicobacter pylori* and *Klebsiella pneumoniae*.

61. The library of claim 60 wherein said library of compounds exhibits antibacterial activity by binding to a pilus chaperone thereby inhibiting pili assembly.

62. The library of claim 61 wherein the pilus chaperone is a PapD chaperone.

63. The library of claim 61 wherein the pilus chaperone is a FimC chaperone.

64. A method for monitoring solid-phase synthesis of a compound having the formula:



wherein each of R_1, R_2 and R_3 is independently a substituted or unsubstituted alkyl (C_{1-10}), substituted or unsubstituted acyl (C_{2-15}), substituted or unsubstituted aryl (C_{6-14}), substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl (C_{7-15}), substituted or unsubstituted heteroarylalkyl and substituted or unsubstituted heterocycloalkyl; and

R_4 is a carboxy ($-CO_2H$), carboxamide ($-CONH_2$), aldehyde (-CHO), boronate ($-B(OH)_2$), phosphonate ($PO(OH)_2$) and ketone (-COR) wherein R is a halogenated or unsubstituted alkyl (C_{1-3}), wherein said method comprises:

a. affixing a linker compound of any one of claims 38-41, 43, 45 or 48 onto a solid support;

b. utilizing a means for measuring a signal originating from the linker compound; and

c. utilizing said signal as an internal reference thereby enabling the monitoring of reactions of said solid-phase synthesis of compounds.

65. The method of claim 64 wherein said signal originating from said linker compound is a ^{19}F resonance.

66. The method of claim 65 wherein said means for measuring the ^{19}F resonance is ^{19}F NMR spectroscopy.

67. The method of claim 64 wherein said solid support comprises polystyrene resin beads, silica chips and polyethylene glycol resins.

68. The method of claim 67 wherein said solid support is polyethylene glycol grafted polystyrene resin.

69. A complex comprising the compound of any one of claims 1-20 complexed with a linker compound of any one of claims 38-41, 43, 45 or 48 wherein said linker compound is affixed to a solid support.

Figure 1

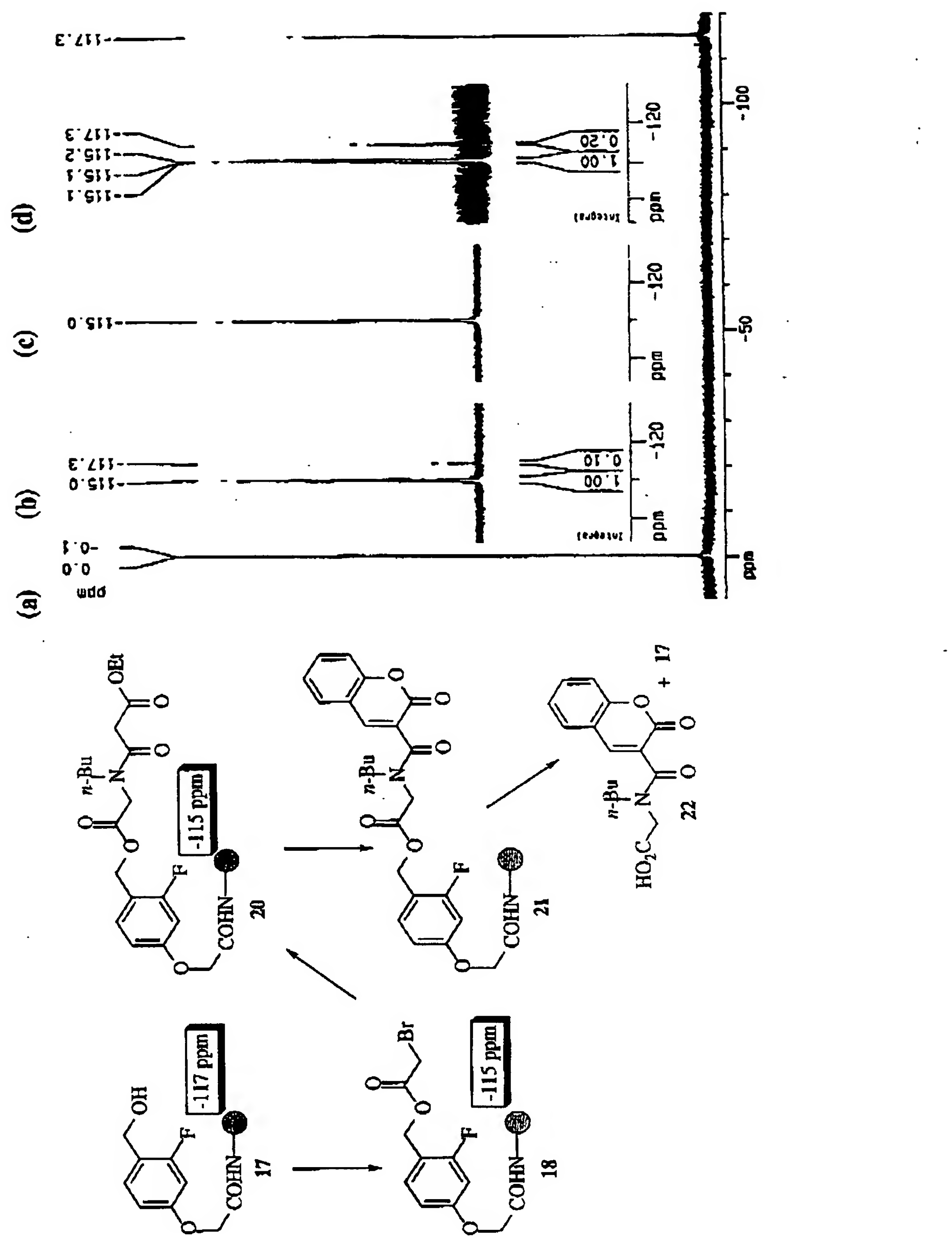


Figure 2

23

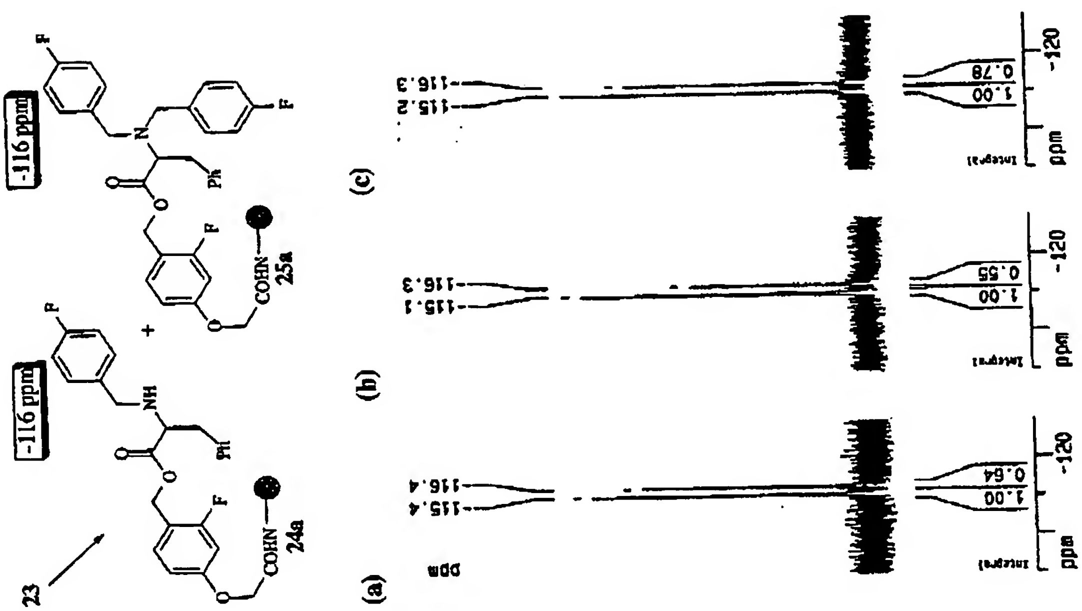

-116 ppm
-116 ppm
NH
N
F
Ph
COHN
24a
25a
(a)
(b)
(c)
ppm
-115.4
-116.4
1.00
0.64
-115.1
-116.3
1.00
0.55
-115.2
-116.3
1.00
0.78
Integral
ppm
-120

Figure 3

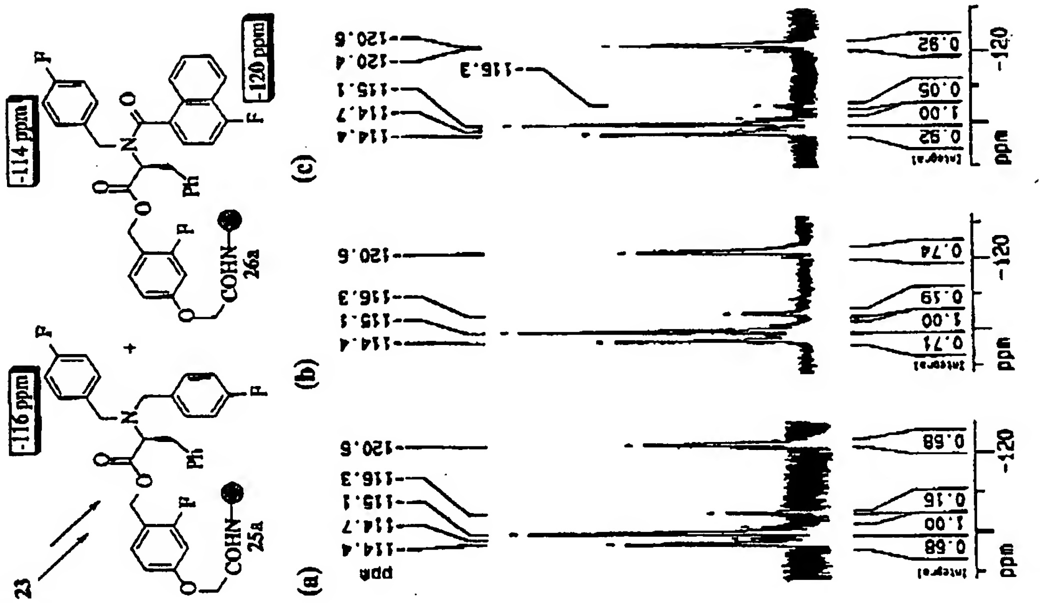
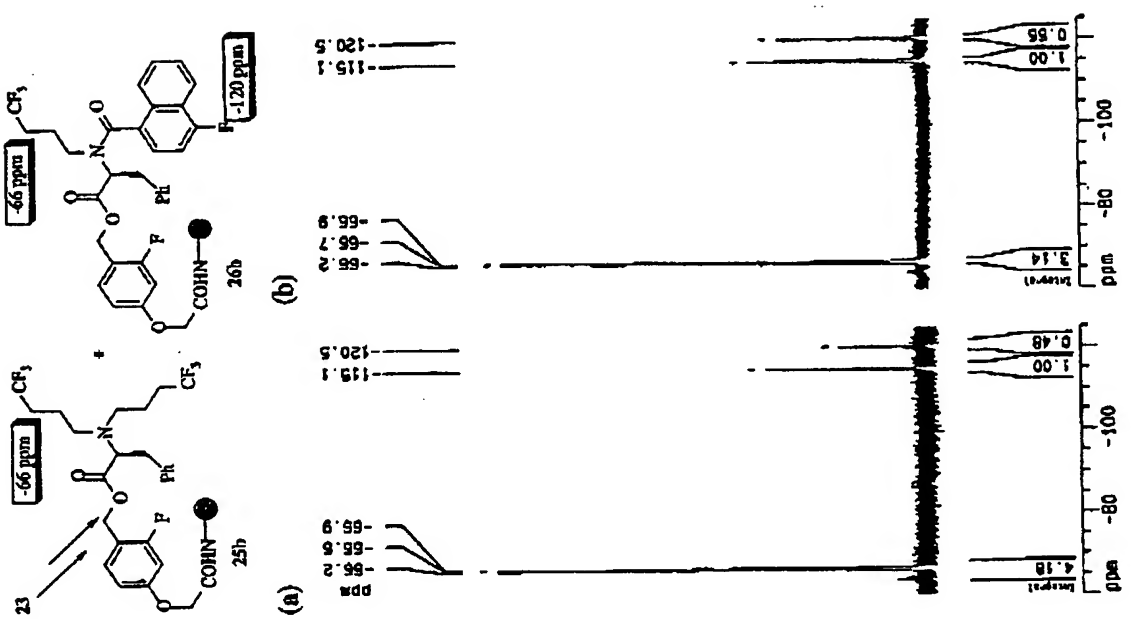


Figure 4


23
-66 ppm
-120 ppm
25b
26b
(a)
(b)
-66.2
-66.5
-66.9
-66.7
-115.1
-120.5
4.18
1.00
0.48
3.14
0.56
-80
-100
ppm

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/26177

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A01N 57/00; C07D 305/00, 309/00, 311/00, 311/02, 311/04, 321/00
US CL :436/518, 536; 514/99, 100; 549/263, 273, 275, 283, 285, 287, 290
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/518, 536; 514/99, 100; 549/263, 273, 275, 283, 285, 287, 290

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

US PTO databases: West, East and STN Express: CASONLINE, CAPLUS, CAOLD, Marpat, Beilstein, EMBASE, Biosis, Medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SVENNSSON et al. Fluorinated Linkers for Monitoring Solid-Phase Synthesis Using Gel-Phase 19F NMR Spectroscopy. Tetrahedron Lett. 24 September 1998, Vol. 39, pages 7193-7196, especially page 7195.	1-26, 31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

07 JANUARY 2001

Date of mailing of the international search report

16 JAN 2001

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/26177

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-26 and 31

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claim(s) 1-26, drawn to a compound of the formula as defined in claim 1 and claim(s)31, drawn to a pharmaceutical composition of claim 23.

Group II, claim(s) 27, drawn to a first method of treating a Gram-negative infection, administering an effective amount of a compound of claim 23.

Group III, claim(s) 28, drawn to a second method of preventing or inhibiting the attachment of a Gram-negative organism to host tissues in a mammal, comprising administering an effective amount of a compound of claim 23.

Group IV, claim(s) 29, drawn to a third method of inhibiting biofilm formation, comprising administering an effective amount of a compound of claim 23.

Group V, claim(s) 30, drawn to a fourth method of preventing or inhibiting bacterial colonization by a Gram-negative organism in a mammal, comprising administering an effective amount of a compound of claim 23.

Group VI, claim(s)32-33, drawn to a process for the preparation of a compound of the formula as defined in claim 32.

Group VII, claim(s)34-41, drawn to a linker of the formula as defined in claim 34.

Group VIII, claim(s)42-45, drawn to a first process of synthesizing a linker compound.

Group IX, claim(s)46, drawn to a compound produced by the process of claim 45.

Group X, claim(s)47-48, drawn to a second process of synthesizing a linker compound.

Group XI, claim(s)49, drawn to a third solid phase process for the preparation of a compound of the formula as defined in claim 32.

Group XII, claim(s)50-52, drawn to a fourth solid phase process for the preparation of a compound of the formula as defined in claim 50.

Group XIII, claim(s)53, drawn to a fifth solid phase process for the preparation of a compound of the formula as defined in claim 32.

Group XIV, claim(s)54-59, drawn to a library of compounds of the formula as defined in claim 54.

Group XV, claim(s)64-68, drawn to a method for monitoring solid-phase synthesis of a compound having the formula as defined in claim 64.

Group XVI, claim(s)69, drawn to a complex comprising a compound as defined in any one claims 1-20 and a linker compound as defined in any one of claims 38-41, 43, 45 or 48.

The inventions listed as Groups I-XVI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features in each of the aforementioned groups for the following reasons:
[1] Group I is drawn to a compound of the formula as defined in claim 1 and to a pharmaceutical composition of claims 23 and 31.
[2] Group II is drawn to a first method of treating a Gram-negative infection, administering an effective amount of a compound of claim 23.
[3] Group III is drawn to a second method of preventing or inhibiting the attachment of a Gram-negative organism to host tissues in a mammal, comprising administering an effective amount of a compound of claim 23.
[4] Group IV is drawn to a third method of inhibiting biofilm formation, comprising administering an effective amount

of a compound of claim 23.
[5] Group V is drawn to a fourth method of preventing or inhibiting bacterial colonization by a Gram-negative organism in a mammal, comprising administering an effective amount of a compound of claim 23.
[6] Group VI is drawn to a process for the preparation of a compound of the formula as defined in claim 32.
[7] Group VII is drawn to a linker of the formula as defined in claim 34.
[8] Group VIII is drawn to a first process of synthesizing a linker compound.
[9] Group IX is drawn to a compound produced by the process of claim 45.
[10] Group X is drawn to a second process of synthesizing a linker compound.
[11] Group XI is drawn to a third solid phase process for the preparation of a compound of the formula as defined in claim 32.
[12] Group XII is drawn to a fourth solid phase process for the preparation of a compound of the formula as defined in claim 50.
[13] Group XIII is drawn to a fifth solid phase process for the preparation of a compound of the formula as defined in claim 32.
[14] Group XIV is drawn to a library of compounds of the formula as defined in claim 54.
[15] Group XV is drawn to a method for monitoring solid-phase synthesis of a compound having the formula as defined in claim 64.
[16] Group XVI is drawn to a complex comprising a compound as defined in any one claims 1-20 and a linker compound as defined in any one of claims 38-41, 43, 45 or 48.

The prior art discloses a linker compound as defined by the formula of claim 34 (I) and a compound of the formula as defined in claim 1 (see, Svensson et al. Tetrahedron Lett., Publication Date:24 September 1998, see entire document, especially Schemes I, II and III).
Therefore, Groups I-XVI lack a special technical feature.